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WHOLE BODY AUTORADIOGRAPHY AND FLUOROGRAPHY OF TWO TETRACYCLINE COMPOUNDS IN TUMOUR BEARING MICE

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Abstract 1 The radioactivity and fluorescence distribution pictures of tritium labelled tetracycline in some tumour bearing mice were investigated by means of autoradiography and fluorescence technique applied to whole body sections. In some tissues including blood, bone marrow lung, heart muscle red pulp and marginal zone of the spleen, thyroid, brown fat, stomach (contents) and kidney fluorescence was weaker than would be expected from the concentration of the drug. The reasons for this are discussed A high tissue content of calcium seems to enhance the fluorescence more than can be accounted for only by the concentration. In the von Kossa positive parts of an osteosarcoma fluorescence and to a less extent radioactivity was strong and persistent In the two von Kossa negative soft tumours fluorescence as well as radioactivity was rather weak.

2 After the administration of demethylchlortetracycline to mice bearing ten different kinds of tumours, persisting fluorescence after 24 h was seen only in three osteosarcomas and one mammary carcinoma and was confined to von Kossa positive areas.

Our results favour the view that tetracyclines are retained in tumour tissue with an increased calcium content which is probably less important in increasing the real concentration of the drugs than in enhancing their fluorescence.

In an earlier paper (7) the fluorescence properties of different tetracyclines as well as their distribution in normal tissues were investigated by means of fluorescence technique applied to whole body sections of mice The distribution pattern was compared to that obtained by autoradiography of tritium labelled tetracycline (H^3 TC) A striking similarity between autoradiogram and fluorogram was seen although some differences were also noted However the two techniques were used on different animals which to some extent may explain some of these differences

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In the literature there are many reports on the affinity of tetracyclines for tumour tissue Most of the studies have been made with fluorescence technique using different tetracycline compounds though in a few investigations radioactive tetracyclines were used The only investigation comparing the two techniques is that of Machado et al (21) who found the correlation between radioactivity (estimated by means of tritium assay) and fluorescence intensity of H^3 TC to be surprisingly low

In order to further investigate the validity of fluorescence as a reflexion of the real concentration of tetracycline (TC) in tumours and normal tissues it was thought to be of interest to compare autoradiograms and fluorograms from the same tumour bearing animal and even from identical sections Further to gain some more information on the possible persistence of tetracyclines in tumour tissue a number of mice bearing different kinds of tumours were investigated for fluorescence at 24 h after the administration of the tetracycline compound demethylchlortetracycline (DMCTC)

MATERIAL AND METHODS

H^3 TC was used the specific radioactivity as given by the manufacturer being [illegible] mC/mg (New England Nuclear Corp Boston, Mass) To obtain optimal fluorescence non labelled TC was added as carrier (Achromycin®) The final product had a specific radioactivity of 0.6 mC/mg. It was administered intravenously as a single dose of 4[illegible] mg/kg of body weight to four mice bearing a radiostrontium induced osteosarcoma ("osteoblastic medium") transplanted through four generations (osteosarcomas supplied by Dr A. Nilsson, Research Institute of National Defence Sundbyberg Sweden) The animals were sacrificed at [illegible]0 min, 80 min 3[illegible]0 min and 24 h

Table I *Fluorescence of demethylchlortetracycline in different tumours of mice at 5 and 24 h after injection*

Type of tumour	Induction	Retransplantations	Administration	Fluorescence (5 h)	Fluorescence (24 h)
Fibroblastic osteosarcoma	Radiation	Few	I v	3+	1+
Fibroblastic osteoblastic sarcoma	Radiation	Few	I p	3+	2+
Osteoblastic sarcoma (intense bone formation)	Radiation	Few	I v	No obs	4+
Thymoma SES 3 HT	Polyoma virus	Several	I v	3+	0
Thymoma SECBF	Polyoma virus	Several	I v	2+	0
Mammary carcinoma SECR	Polyoma virus	Several	I v [a]	(+)	0
Mammary carcinoma SECX	Polyoma virus	Several	I p	No obs	3+ [b]
Sarcoma SEC 2 H	*Polyoma virus*	*Several*	I p	2+	0
Fibrosarcoma	Methylchol anthrene	None	I v	2+	0
Primary not identified	Polyoma virus	None	I v	No obs	0

[a] The 100 mg of demethylchlortetracycline were divided into four doses
[b] Fluorescence was observed only in several small spots in the tumour

after injection For comparison with soft non bone forming tumour tissue one mouse with a thymoma (SES-3M) and another mouse with a sarcoma (SESO-C_7) (*soft tumours supplied by Dr O Sjogren Dept of* Tumour Biology Karolinska Institutet Stockholm, Sweden) both induced by means of polyoma virus and transplanted through several generations were sacrificed at 80 min after injection One osteosarcoma bearing animal served as a control to study the autofluorescence of the tissues.

To investigate the persistence of fluorescence in experimental tumours another series of mice were used bearing ten different tumours comprising three radio strontium-induced osteosarcomas with different degree of bone formation six soft tumours induced by means of polyoma virus and one soft tumour induced by means of methylcholanthrene (Table I) In this study DMCTC (Ledermycin®) was used since the fluorescence of this compound has proved to be less pH-dependent and possibly to have a more suitable colour (orange yellow) than others of the tetracycline group (7) It was administered intravenously or intraperitoneally in a dose of *100 mg/kg of body weight, and the mice were killed at* 5 h and 24 h after injection

Tape sectioning technique

Killing of the mice was accomplished by immersion into a mixture of solid CO and acetone (−75 C) after ether anaesthesia. At −10 C sagittal sections through the animals were cut according to the tape sectioning technique described by Ullberg (for references see (7)) the thickness of the sections being 60 μ which has proved suitable for fluorescence purposes

Fluorescence autoradiography and staining techniques

After drying in the freeze room the sections were photographed under UV light using the method described in a previous communication (7) (light source Philips HPW 125 W photographic registration obtained on Kodachrome X daylight colour film through a Kodak Wratten 2 E filter) As reported it is possible to convert colour photographs into black and white paper prints by *rephotographing them through a monochromatic filter* mainly transmittable for yellow light This procedure however has proved incapable of excluding all non yellow light and thus in the present work the estimations of fluorescence intensity were always made from the original colour photographs The photographed sections from the animals given H^3 TC were used for autoradiography *according to the method of* Ullberg (exposure obtained by apposition against Ilford G5 nuclear emulsion plates time of exposure four months) Finally the sections were stained for calcium by means of the von Kossa technique

RESULTS

Comparison between the fluorescence and autoradiographic distribution pictures of tritium labelled tetracycline

Fluorograms and autoradiograms of mice killed at 20 min 80 min and 24 h after injection of H^3 TC are shown in Figs 5 and 6 In many organs there is a close correlation between fluores



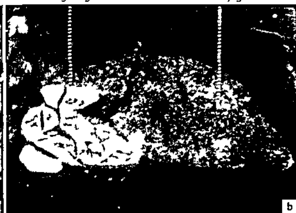


Fig 1 (*a*) Detail autoradiogram showing higher uptake of H TC in submaxillary gland than in sublingual gland at 0 min after injection (*b*) Detail autoradiogram showing higher uptake of H TC in sublingual gland than in submaxillary gland at 80 min after injection The concentration in the sublingual gland is also higher than at 0 min.

cence and radioactivity However in several tissues little or no fluorescence is observed in spite of the fact that H³ TC is obviously present as judged from the autoradiograms These tissues include the *blood bone marrow lung heart muscle spleen* (*red pulp and marginal zone*) *thyroid brown fat stomach* (*contents*) and *kidney*

At 20 min and 80 min the *liver* shows maximal radioactivity as also do small areas in *bone* and *teeth* although the fluorescence of the liver is not so strong as that of the latter organs Thus in bone and teeth the fluorescence of H³ TC is stronger than would be expected from its concentration as judged from the autoradiograms This is still more so in the mineralizing parts of the *osteosarcoma* where maximal and persisting fluorescence is seen although the radioactivity of this tissue is lower than for example in the liver The only soft organ behaving in a similar way is the *sublingual gland* which at 20 min exhibits as strong fluorescence as the submaxillary gland in spite of much lower radioactivity



Fig Detail autoradiogram of thyroid and parathyroid at 0 min after injection of H TC Note high uptake in parathyroid gland

Another finding regarding the salivary glands is that at 80 min the sublingual gland has the higher radioactivity of the two glands and also a higher radioactivity than at 20 min (Fig 1)

Some organs may need some further notice The *parathyroid gland* accumulates H³ TC to a remarkable extent at 20 min (Fig 2) and 80 min but is quite devoid of fluorescence and radioactivity at 24 h The *brain testis* and *thyroid colloid* never show fluorescence or radioactivity *Fat* and *fibrous cartilage* (intervertebral discs) never fluoresce but initially show slight radioactivity In the remaining soft organs fluorescence fairly well parallels radioactivity with various degrees of initial uptake and due decay at 320 min and 24 h The *bone* and *teeth* still exhibit persisting maximal intensity of fluorescence and radioactivity 24 h after injection Somewhat lower and less persisting fluorescence and radioactivity is seen in *hyaline cartilage* (somewhat higher in tracheal than in articular cartilage)





Fig 3 (*a*) Autoradiogram showing the distribution of H TC in a bone forming tumour (osteoblastic medium) at 80 min after injection (*b*) Corresponding section of the autoradiogram in Fig 3*a* stained according to the von Kossa technique The uptake of H TC in Fig 3*a* corresponds to areas of high calcium content

In the osteosarcoma persisting very strong fluorescence and rather high radioactivity is seen in the mineralized parts (as judged from the von Kossa stained section) as mentioned above (Fig 3) The haemorrhagic parts of the tumour do not contain H^3 TC whereas the peripheral parts without signs of bleeding and with little or no calcification show a rather high uptake at 20 min but lose it at approximately the same rate as most organs In the *soft tumours* which are von Kossa negative (Fig 4) a concentration of H^3 TC is seen which approximately amounts to that of the lymphatic organs but by no means reaches that of the skeleton or the excreting organs (Fig 7) Only one mouse bearing each kind of soft tumour was available and was sacrificed at 80 min after injection and thus an estimation of the possible persistence of H^3 TC at 320 min and 24 h could not be made The intensity of radioactivity slightly exceeds that of fluorescence in both soft tumours

The persistence of demethylchlortetracycline induced fluorescence in ten different tumours of mice

The types of tumours are listed in Table I The fluorescence intensity has been divided into six degrees (4+ = very strong 3+ = strong 2+ = moderate 1+ = weak (+) = doubtful 0 = no fluorescence) At 5 h after injection all tumours observed show fluorescence of varying intensity At 24 h the osteosarcoma with intense bone

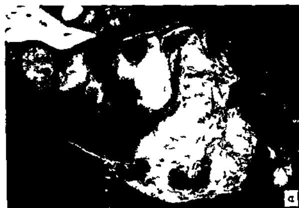




Fig 4 Autoradiographic distribution of H TC in a soft tumour (thymoma) at 80 min after injection (*a*) and the corresponding von Kossa stained section (*b*) Note rather low uptake of H TC and absence of calcium

formation exhibits strong and homogenous fluorescence the other osteosarcomas also show considerable fluorescence but only in rather small spots One mammary carcinoma also shows spotted fluorescence at 24 h the other soft tumours being completely devoid of fluorescence The four tumours with fluorescence at 24 h (that is three osteosarcomas and one mammary carcinoma) are the only ones to show positive von Kossa stain and the fluorescing parts and the von Kossa positive parts of the tumours coincide exactly (Fig 8) This may indicate the presence of calcium in those areas where persisting fluorescence is seen

DISCUSSION

Tissue fluorescence and radioactivity of tritium labelled tetracycline

In many tissues good correlation is seen between the fluorescence and radioactivity of H^3 TC (Figs 5 6 and 7)

However in several tissues or organs fluorescence *is weaker than would be expected from* the concentration of the compound Thus in the *blood* no fluorescence is seen which is in keeping with earlier observations that blood pigment quenches the fluorescence not only of tetracyclines but also of other compounds The richness of blood may explain why very little or no fluorescence is exhibited also by the *bone marrow lungs red pulp and marginal zone of the spleen* and *heart muscle* (in contrast to skeletal muscle) In some of these tissues fluorescence has been demonstrated by other authors (e g 8) by means of ultraviolet microscopy which obviously makes it possible to observe fluorescing tissue between the erythrocytes In the *brown fat* the quenching theory" may possibly also hold true since the cells of this tissue likewise contain a pigment though of unknown composition The *thyroid gland* never shows gross fluorescence it also seems to be quite devoid of radioactivity but at high magnification it can be seen that it is only the colloid which does not contain H^3 TC the narrow cellular areas showing weak radioactivity (Fig 2) It seems possible that these areas are too small to exhibit gross fluorescence and that they perhaps would fluoresce under the UV microscope The *stomach contents* show rather strong radioactivity but no fluorescence an explanation would be their acidity since the fluorescence of tetracyclines diminishes or disappears at an acid pH This factor may be of importance also in the *urine* the pH of which is about 5–6 in mice fed a standard diet The relatively weak fluorescence of the *kidney* may be due partly to the acidity of the urine and partly to the high blood content of this organ

The *mineralized parts of the osteosarcoma* and to a less extent *bone* and *teeth* exhibit stronger fluorescence than would be expected from their concentration of H^3 TC The only soft organ behaving in a similar way is the *sublingual gland* in which at 20 min strong fluorescence but rather low radioactivity is seen it is also more stained by the von Kossa method than almost all other soft tissues which may indicate a higher calcium content However no persisting fluorescence at 24 h is seen in this organ in contrast to the above mentioned hard tissues

The sublingual gland which is a mucous salivary gland is also a unique organ in showing higher radioactivity at 80 min than at 20 min

The only report so far of an accumulation of a tetracycline compound in the *parathyroid gland* seems to be that of Andre in 1956 who found a high uptake of H^3 TC at 30 min after injection (2) Our autoradiograms confirm this finding showing a high concentration at 20 min (Fig 2) and still rather high at 80 min The fluorescence seen on the colour photographs appears somewhat weaker probably because of the very small size of the organ The parathyroid was not present on the sections from the 320 min mouse but at 24 h it is completely devoid of fluorescence and radioactivity Anyhow the fact remains that H^3 TC is strongly taken up by an organ known to regulate calcium metabolism

The affinity of tetracycline compounds for tumour tissue

A rather abundant literature on this subject has appeared during the last years The majority of the investigations have been performed with the aid of the macroscopic or microscopic fluorescence of tetracyclines A much discussed question has been to what components of the tumour tissue the drugs are bound There seem to be three main opinions in this respect some investigators maintain that the fluorescence is seen only in macrophages and cellular debris (1 26)

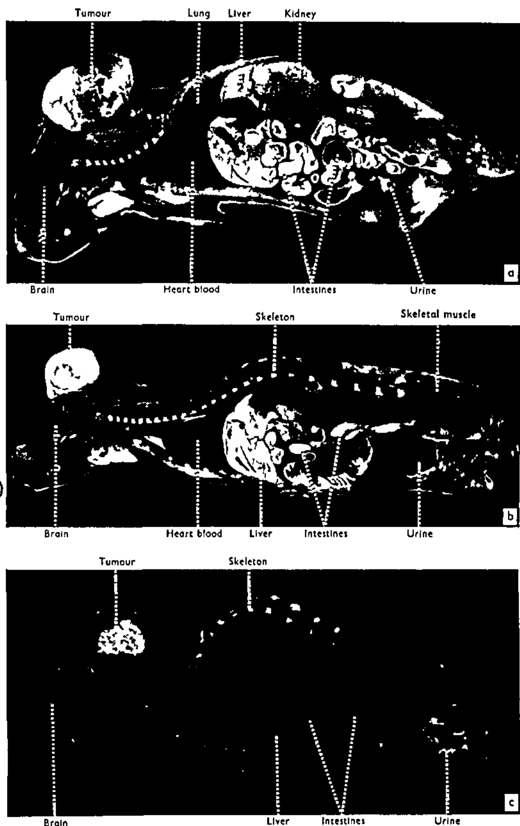
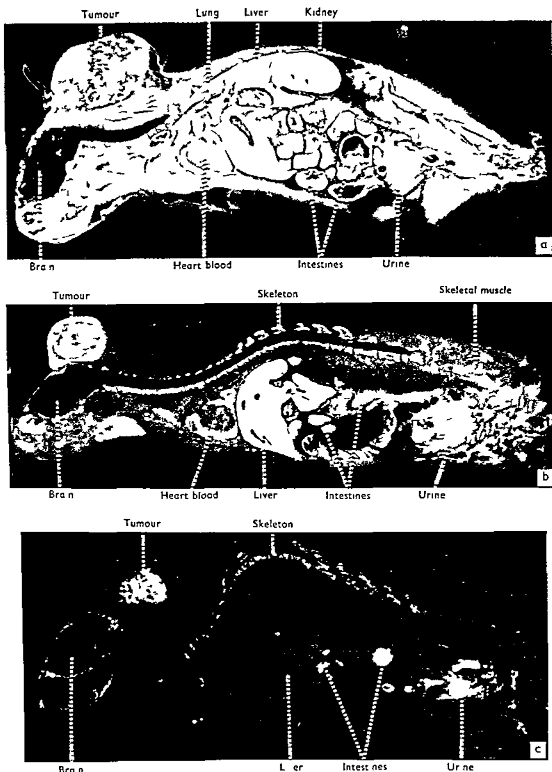


Fig. 5. Whole body autoradiograms of mice with a bone forming tumour (osteoblastic medium) killed at 20 min (*a*), 80 min (*b*) and 24 h (*c*) after the injection of H TC. See text for details.



F g 6 Fluorescence of H TC exhibited by the sections from which the autoradiograms in Fig. 5 were obtained (*a* 0 min *b* = 80 min and *c* = 4 h after injection) The organs w th the strongest fluorescence have a whitish yellow colour The spontaneously red fluorescing organ behind the eye is the Harderian gland of rodents. See text for de ails

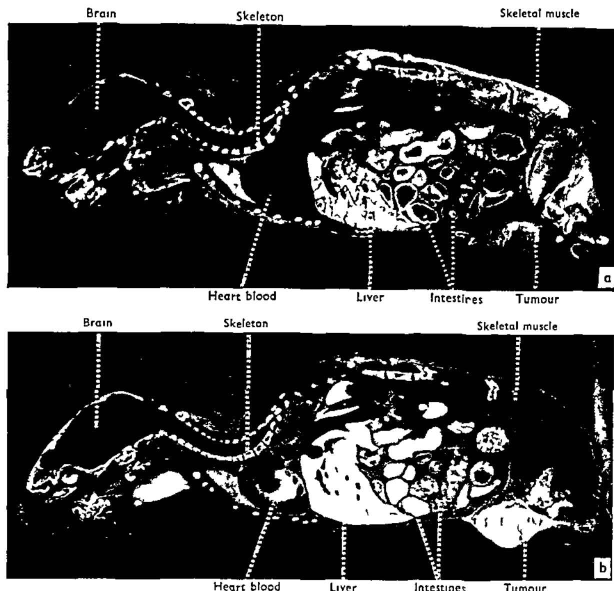


Fig 7 Fluorogram (*a*) and autoradiogram (*b*) from a mouse with a soft tumour (sarcoma) at 80 min after the injection of H TC Note rather weak fluorescence and low radioactivity in the tumour

others that it is found within live tumour cells (e g 4) and still others that tetracyclines are ac cumulated or at least retained only in tumour cells undergoing necrosis in which the presence of calcium is regarded by some to play a part

Findings supporting the latter theory have been reported by many authors Donsky (10) macro scopically estimated the fluorescence to be located in necrotic or granulomatous areas of skin car cinomas Barlow et al (5) found fluorescence in calcified incrustations of bladder carcinomas and only rarely within the tumour tissue itself Engel bart et al (13) observed lasting fluorescence only in grossly necrotic areas of 33 tumours of mice rats hamsters and rabbits Machado et al (21) found persisting fluorescence in sarcomas 37 and Ehrlich solid tumours of mice fluorescence was confined to tumour cells in different stages of degeneration while none was seen in well pre served tumour cells in zones of advanced necrosis with indistinct cell morphology or in the stroma Very similar results have b en obtained by Tapp et al (29) in squamous skin carcinomas of rab bits and in ovarian tumours mammary tumours





Fig 8 Persisting fluorescence of DMCTC (*a*) in the von Kossa positive areas (*b*) of a mammary carcinoma at 4 h after injection.

and stem cell leukemias of rats The latter authors further induced necroses in initially non necrotic non fluorescent mammary carcinomas by ligation of the arteries and afterwards found fluorescence in cells undergoing necrosis but not in live tumour cells nor in dead structureless areas devoid of circulation They also succeeded in staining necrotic parts of tumours with TC in vitro (30) and found the uptake to be highest in areas showing a positive von Kossa stain although somewhat weaker fluorescence was seen also in the von Kossa negative areas in both these types of necrotic tissue fluorescence could be prevented by pretreatment with Na EDTA

Somewhat puzzling results in this respect are reported by Riley et al (25) They agree that TC is bound to calcifications within tumour cells but have been unable to find any signs of necrosis even with the aid of electron microscopy This is at variance with earlier findings that the calcium content of live tumour tissue is generally lower than normal (9 28) but rises to abnormally high levels when necrosis begins (28)

A considerable amount of evidence however has been put forward in recent years that tetracyclines are fixed in necrotic cells of many kinds irrespective of the type of cell or of what lies behind the necrosis Malek et al have studied the fixation of tetracyclines (mostly chlortetracycline CTC) in experimentally induced ischaemic foci of canine myocard (22) This fixed CTC is said to differ from CTC harbouring in non damaged tissue in having a stronger yellow colour and being resistant to boiling alkali and formol fixation The ischaemic foci have been found to be von Kossa positive and to take up increased amounts of radiocalcium (22) Tapp Carroll and Kovacs (30) have demonstrated the retention of TC in necrotic cells of different tissues in addition to the experimental tumours mentioned above whether the origin of the necrosis is ischaemia or various toxic drugs They found the fluorescence to be strongest in von Kossa positive areas and since it is known that necrotic parts of the liver and of tumours (28) contain increased amounts of calcium they conclude that tetracyclines may be retained in necrotic cells through binding to calcium The fact that also von Kossa negative necrotic tissue may exhibit some fluorescence might mean that tetracycline fluorescence is more sensitive for demonstration of calcium than the von Kossa technique

Though unproved a similar mechanism might possibly lie behind the fixation of tetracyclines in an additional number of pathologically altered tissues including bronchopneumonic and pyelonephritic foci inflammatory necroses subcutaneous granulomas and cutaneous microabscesses skin ulcers stomach ulcers necroses of kidneys and livers necrotic areas after liver resection torn skeletal muscle burns cadmium induced degenerative changes of the testes and cells damaged by virus in vitro

The calcium hypothesis gains further support from the fact that tetracyclines are chelating agents (15) forming complexes with certain metal ions such as calcium and zinc This could account for the fixation of tetracyclines in virtually all

tissues normal or pathological where gross calcification is known to occur (e g 1 11 13 16 27) Moreover it has been shown that tetracyclines form precipitates with beta lipoproteins only in the presence of calcium (6) and are able to bind macromolecules (DNA and albumin) through the mediation of calcium and zinc (18)

To these findings can be added the work of Finerman and Milch (15) who considered the persistence of tetracycline fluorescence in biological tissues to be directly related to the calcium content of the tissue probably by the interaction with calcium ions in "hydroxyapatite seeded nucleation sites on collagen fibrils Loo et al (20) in 1957 without taking calcium into account maintained that in sarcoma 37 of mice TC is bound by forming a complex with a peptide it possibly could be suggested then that this complex would be mediated by calcium

It is also to be noted that other compounds than tetracyclines are known to localize in tumours and that some of these also accumulate in bone for example some of the porphyrins (3 19) and the dye Alizarin red S (21)

Our own experiments on tumours of mice have shown lasting fluorescence of DMCTC only in areas of high calcium content as indicated by the von Kossa method However we only used a single dose of 100 mg/kg of body weight which although rather high if the LD$_{50}$ is taken into account is less than that of most investigators (13 21 24 29) Thus it may be possible to obtain fluorescence even in tissues without histological signs of calcification if higher doses are used (24)

CONCLUSIONS

A high and persisting uptake of H^3 TC and DMCTC in osteosarcomas and soft tumours of mice was found to coincide exactly with the von Kossa positive parts of the tumours whereas sometimes a rather high initial accumulation but no retention was seen in von Kossa negative areas This is in agreement with the findings of many if not all other authors that tetracycline induced fluorescence in tumour tissue as well as in other kinds of pathological tissue may be related to the calcium content of the tissue

Our experiments further seem to confirm that the fluorescence of TC does not quite mirror its concentration Gross fluorescence is diminished or extinguished by high amounts of blood and by an acid pH and in addition the fluorescing structures may of course be too small to be detected macroscopically

On the other hand a high tissue content of calcium enhances the fluorescence intensity more than can be accounted for merely by the increased concentration of the drug This is true of normal as well as tumour tissue and it is in keeping with the results of Machado et al (21) who investigated the distribution of H^3 TC in tumours of mice by means of tritium assay and fluorescence technique They found much higher concentrations of H^3 TC necessary to induce fluorescence in for example liver or heart muscle than in the necrotic parts of the tumours assumed to have an increased calcium content Further the fluorescence of aqueous solutions of tetracyclines is highly increased by the addition of calcium (or other divalent metal ions) and the fluorescence of tetracyclines in tumour tissue can be prevented by previous treatment with Na EDTA Moreover the relatively few investigations performed with radioactive tetracycline compounds have given no unequivocal evidence of the retention of radioactivity in soft tumours (12 14 17 21 23) whereas fluorescence investigations have yielded more positive results Eskelson et al (14) even found other radioactive compounds to be retained in a mouse tumour to the same extent as different radio-iodinated tetracyclines

It is concluded that persisting fluorescence of tetracyclines in tumours as in other tissues can be ascribed to the presence of calcium which is probably less important in increasing the real concentration of the drugs than in enhancing their fluorescence

REFERENCES

1 Ackerman M B & McFee A S Tetracycline fluorescence in benign and malignant tissues Surgery 53 47 1963
2 André T Studies on the distribution of tritium labelled dihydrostreptomycin and tetracycline in the body Acta radiol (Stockh) Suppl 118 1956
3 Armstrong W D Engstrom A & Paul K G Porphyrins and bone S and J clin Lab Invest 14 7 196
4 Ayre J F LeGuerrier J & Arsenault J Tetracycline induced autofluorescence in neoplastic cells. Med Tms (N Y) 93 885 1965

5 Barlow K. A Maurice B A & Atkins P Ultra violet fluorescence of bladder tumours followin, oral administration of tetracycline compounds a macro scopic microscopic and fluorescence spectrophotometric study Cancer (Philad) 19 1013 1966
6 Ber quist L M Janzen C L Simm N M & Searcy R L Formation of lipoprotein tetracycline complexes in human serum Antimicrobial Agents and Chemotherapy 3 477 1963
7 Blomquist L & Hanngren A Fluorescence technique applied to whole body sections for distribution studies of tetracyclines Biochem Pharmacol 15 215 1966
8 Bottiger L E On the distribution of tetracycline in the body Antibiot and Chemother 5 33 1955
9 Braunschweig A Dunham L J & Nichols S Potassium and cal ium content of gastri carcinoma Cancer Res 6 230 1946
10 Donsky H J Tetracycline fluorescence in squamous cell carcinoma Arch Derm 9? 388 1965
11 Douglas A C The deposition of tetracycline in human nails and teeth a complication of lon term treatment Brit J Dis Chest 57 44 1963
1 Dunn A L Eskelson C D McLeay J F O, born R E & Walske B R. Preliminary study of a radioactive product obtained from iodinating tetracycline Proc Soc exp Biol (N Y) 104 1? 1960
13 Engelbart K Gericke D & Soder A Tierexperimenteller Beitrag zur Frage der Einlagerung von Tetracyclin in Tumorgewebe Z Krebsforsch 66 310 1964
14 Eskelson C D Dunn A L Osborn R E & McLeay J F Distribution of some radioiodinated tetracyclines in animals J nucl Med 4 38? 1963
15 Finerman G A M & Milch R A In vitro binding of tetracyclines to calcium Nature (Lond) 198 486 1963
16 Hakkinen I & Lindgren I The localization of tetracycline in the metastatic calcifications in the stomach of rat induced by overdosage of dihydro tachysterol and vitamin D Acta path microbiol scand 59 4 8 1963
17 Hlavka J H & Buyske D A Radioactive 7 iodo 6 deoxytetracycline in tumour ti sue Nature (Lond) 186 1064 1960
18 Kohn K W Med ation of d valent metal ions in the bindin, of tetracycline to macromolecules Nature (Lond) 191 1156 1961
19 Lipson R L Pratt J H Baldes E J & Dockerty M B Hematoporphyrin deri ative for detection of cervical cancer Obstet and Gynec 4 78 1964
0 Loo T L Titus E D & Rall D P Nature of fluorophore local zin, n tetracycl ne treated mouse tumor Science 1 6 ,53 1957
1 Machado L Zaidman I Gerstein J F Lichtenberg F & Gray S J Factors affectin, the site and degree of localizat on of tetracycline in sarcoma 37 tumors Can er Res 24 1845 1964
– Malek P Hammer J Zastava V & Pisa Z. The diagnostic s gnif cance of fixation of tetracycline antibiotics in infarcted myocardium J cardiovasc Surg (Torino) 61 8 1965
23 M Leay J F Walske B R & Osborn R E Tetracycline in tumor Surg Forum 11 79 1960
4 Rall D P Loo T L Lane M & Kelly M G Appearance and persistence of fluorescent material in tumour tissue after tetracycline administration J nat Cancer Inst 19 79 1957
,5 Riley L H Paschall H A & Robinson R A Intracellular calcification occurring in a transplanted human tumor J surg Res 6 171 1966
6 Sherman H H Chryssanthou C Sukoff M H Minnberg D Beckman E M & Weingarten M Tetracycline fluorescence in the diagnosis of gastric carcinoma Gastroenterology 45 84 1963
27 Storey E Tetracycline antibiotics and their effects on calcified and non-calcified ti sue Aust Ann Med 1 3?5 1963
?8 Suntzeff V & Carruthers C Potassium and calcium in ep dermal carcino-genesis induced by methyl cholanthrene J biol Chem. 153 5 1 1944
?9 Tapp E Carroll R & Kovacs K Tetracycline fluorescence in experimental tumours Brit J Cancer 19 538 1965
30 Tapp E Kovacs K & Carroll R Tetracycline staining of tissues in vitro Sta n Technol 40 199 1965

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REFERENCES

1 Ackerman M B & McFee A S Tetracycline fluorescence in benign and malignant tissues Surgery 53 47 1963

André T Studies on the distribution of tritium labelled dihydrostreptomycin and tetracycline in the body Acta radiol (Stockh) Suppl 118 1956

3 Armstrong W D Engstrom A & Paul K G Porphyrins and bone Scand J clin Lab Invest 14 7 196

4 Ayre J E LeGuerrier J & Arsenault J Tetracycline induced autofluorescence in neoplastic cells Med Tms (N Y) 93 885 1965

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COMPARISON OF THE DISTRIBUTION OF DEMETHYLCHLORTETRA CYCLINE AND RADIO CALCIUM IN WHOLE BODY SECTIONS OF TUMOUR BEARING MICE

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Abstract The fluorescence distribution of demethylchlor tetracycline (DMCTC) and the autoradiographic distribu tion of Ca^{45} have been stud ed in whole body sections of mice with mammary carcinomas High and persisting accumulation of both compounds is found only in cal cified organs such as bone teeth and tracheal cartilage Though no gross calcification is present in the tumour as judged from the von Kossa stained sections Ca^{45} is concentrated in the cellular tumour parts at 1 h and 5 h after injection with a decay at 12 h only weak radio activity is seen in totally necrotic structureless areas DMCTC induced fluorescence seen only in the cellular areas is neither more intense nor retained for a longer time than in many other soft tissues

When both DMCTC and Ca are given simultaneously they interfere with each other s distribution and are for example highly accumulated in the lungs and red pulp of the spleen which suggests an intravascular complex formation This may poss bly indicate a risk of clinical side effects if tetracyclines and calcium are administered simultaneously

Since the discovery by Rall et al (12) in 1957 of the localization of tetracycline in tumour tissue a large number of publications on the subject have appeared Most investigators have been of the opinion that tetracycline compounds do have some kind of affinity for malignant tissue However very few authors have been able to find a constant and reliable uptake of the drugs in n o lasms and some have been very critical re ding the diagnostic value of the method (1 9 19 20) A much discussed question has been to at components in th t mour tetracyclines are ound Some investigat rs maintain that per fluorescence is een only in mac ophages cellular debris oth rs that it is observed within live tumour cells and still others that it is confined to tumour cells subject to necrosis In favour of the latter theory is the fact that tetra cyclines have been shown to be retained in a variety of non malignant necrotic tissues as well and the phenomenon has been ascribed to the increased amounts of calcium in such tissues (3 11 16 17) A more thorough discussion of these matters and a review of the literature were given in an earlier paper in which also our own animal experiments were reported which favour the cal cium hypothesis (5)

The subject of the present work has been to compare the distribution pictures of radio calcium and a tetracycline compound in tumour bearing mice by means of autoradiography and fluores cence technique respectively applied to whole body sections

MATERIAL

Ca (obtained from The Radiochemical Centre Amer sham England) was used for autoradiographic purposes and demethylchlortetracycl ie (DMCTC) (Ledermycin®) for the fluorescence stud es Nine mice bearing a mam m ry carc noma (m ce with mammary carcinoma SECX suppl d by Dr O Sjogren Department of Tumour Bio lo v Karolin k I st tutet Stockholm Sweden) induced by m ns of polyoma v rus and transplanted through several gene ations were divided into three equal groups Three mice were given C a a dose of 750 μC/kg of body we ght A other thr e m ce rece ved 100 mg of DMCTC kg of body we ght To the three remaining m ce were adm n tered fir t D ICTC and immediately after ward Ca in the same dose as to the other animals All admini trat on wa n r ven One mouse from each group was killed at 1 h ano r r at 5 h and the third at 12 h after inj t on

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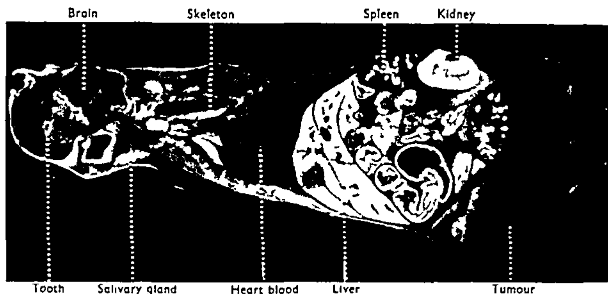


Fig. 1. Fluorescence distribution of DMCTC at 1 h after injection. Note strong fluorescence in the skeleton, teeth, liver, kidney, salivary gland, and white pulp and marginal zone of the spleen, and weak fluorescence in the tumour.

METHODS

Killing of the mice was accomplished by immersion into a mixture of solid CO_2 and acetone (−75 C) after ether anaesthesia. At −10 C sagittal sections through the animals were cut according to the tape sectioning technique of Ullberg (18). The section thickness was 60 μ for fluorescence purposes and 20 μ for autoradiography. In the mice given both DMCTC and Ca^{45} one 60 μ section and one immediately adjacent 20 μ section were taken at each level. After drying in the freeze room the 60 μ sections were photographed under UV light (light source: Philips HPW 125 W; photographic registration obtained on Kodachrome X daylight colour film through a Kodak Wratten 2 E filter) and the 20 μ sections were used for autoradiography according to the method described by Ullberg (18) (exposure obtained by apposition against X-ray film (Structurix, Gevaert); time of exposure 15 days). Finally, some sections from each animal were stained for calcium according to the von Kossa method.

RESULTS

Fig. 1 shows the fluorographic distribution pattern of DMCTC at 1 h after injection. According to fluorescence intensity at 1 h, 5 h and 12 h after injection the organs can roughly be divided into four groups.

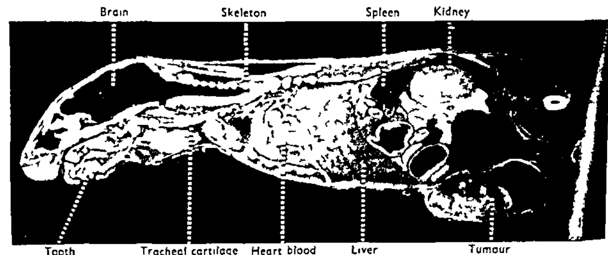


Fig. 2. Autoradiographic distribution of Ca^{45} at 1 h after injection. Note high uptake in the skeleton, teeth, tracheal cartilage, intestine and cellular parts of the tumour.

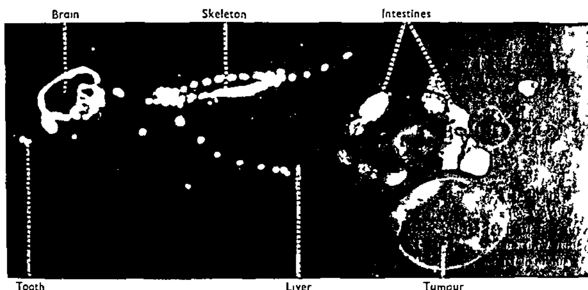


Fig 3 Autoradiographic distribution of Ca at 12 h after injection Note high concentration in bone teeth and intestine

A *Strong and persistent* fluorescence is seen in bone teeth and tracheal cartilage *Strong initial* but somewhat decreasing fluorescence is seen in the excreting organs liver gallbladder and kidney

B *Fairly strong initial* but rapidly decaying fluorescence is observed in the salivary glands lymph nodes thymus marginal zone and white pulp of the spleen and epididymis

C *Weak initial* and rapidly decaying fluorescence is seen in the lungs heart muscle skeletal muscle stomach wall intestinal wall and contents and red pulp of the spleen The pancreas shows p rsistent weak fluorescence

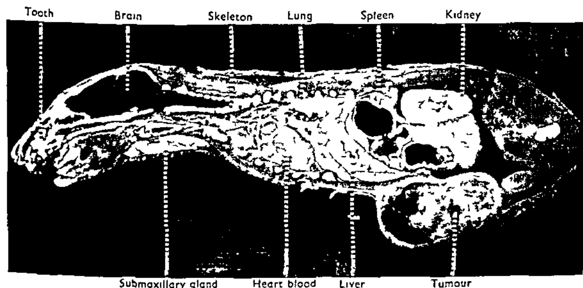


Fig 4 Autorad ogram showing the d str bution of Ca⁴⁵ at 1 h after the injection DMCTC had been gi en immed ately before the Ca A hi her concentration is seen n the l ver kidney spleen submaxillary gland d e pec ally n the lung than is obtained w th only Ca (Fig 2)

D) *No* fluorescence is seen in the blood, CNS and teeth.

The cellular parts of the tumour have a moderate diffuse and low continuous fluorescence, whereas the necrotic, structureless areas are completely non-fluorescent.

Figs. 2 and 3 show the autoradiographic distribution of Ca^{45} at 3 h and 12 h after injection. In a similar way as above the organs have been divided into four groups according to the darkening intensity on the autoradiograms from the mice killed at 1 h, 3 h and 12 h after injection.

A) *High and lasting* uptake is seen in bone, teeth and tracheal cartilage. *High initial* concentration with slow decrease after 12 h is observed in [illegible]

B) [illegible]

C) [illegible]

D) [illegible]

[illegible]

DISCUSSION

The fluorographic distribution of DMCTC in normal tissues is similar to that found earlier (4). However, our experiments with tritium-labelled tetracycline (TC) have shown that the fluorescence of this compound in different organs does not quite reflect its real concentration (5) and DMCTC may well behave similarly in this respect. Thus in the blood, lungs and heart muscle, where haemoglobin quenches fluorescence, the accumulation of DMCTC is probably higher than indicated by the fluorescence intensity. In the kidneys the fluorescence of DMCTC, because of its higher resistance to acidity, probably [illegible] more closely than is the case with the fluorescence of TC (5).

The autoradiographic distribution of Ca^{45} [illegible]

[illegible]

strong fluorescence only in the von Kossa positive parts of hard and soft tumours of mice after the same dose of DMCTC as in the present work (5) It is known that the calcium content of live tumour tissue is lower than normal (6 7 8 15) but rises to abnormally high values when the tissue becomes necrotic (15) Since in the tumours described in the present investigation large areas are totally necrotic and structureless and since certain parts of the cellular areas take up high amounts of calcium it may be assumed that the cells in these parts of the tumours are undergoing necrosis which has not yet reached such an advanced stage as in the structureless areas However fluorescence is low or very low even in these parts it is possible though that stronger fluorescence would have been obtained with higher doses than 100 mg of DMCTC/kg of body weight which is less than most other investigators have required to get lasting fluorescence (10 12 13 14 21)

When both DMCTC and Ca^{45} are given they obviously interfere with each other s distribution Ca^{45} is now seen in organs where normally DMCTC but not Ca^{45} accumulates e g in the liver kidney and lymphatic organs The high accumulation of Ca^{45} in the lungs and red pulp of the spleen seems to indicate an intravascular formation of a complex with rather high molecular weight between Ca^{45} and DMCTC since large molecules such as colloids are known to get trapped in these organs The lungs and red pulp of the spleen likewise exhibit stronger fluorescence of DMCTC when Ca^{4} is added which can be explained either by increased concentration of DMCTC and/or by increased degree of complex formation with calcium which is known to enhance the fluorescence of tetracyclines (5)

The risk of intravascular precipitate formation of such magnitude as to cause multiple occlusions in the capillaries of patients given calcium and tetracycline compounds simultaneously cannot be ruled out especially if both drugs are administered intravenously

REFERENCES

1 Ackerman M B & McFee A S Tetracycline fluorescence in benign and malignant tissues Surgery 53 47 1963

2 Appelgren L E Ericsson Y & Ullberg S A comparison of the distribution of fluorine and calcium by use of double isotope autoradiography Acta physiol scand 53 339 1961

3 Barlow K A Maurice B A & Atkins P Ultraviolet fluorescence of bladder tumours following oral administration of tetracycline compounds a macroscopic microscopic and fluorescence spectrophotometric study Cancer 19 1013 1966

4 Blomquist L & Hanngren A Fluorescence technique applied to whole body sections for distribution studies of tetracyclines Biochem Pharmacol 15 215 1966

5 — Whole body autoradiography and fluorography of two tetracycline compounds in tumour bearing mice Acta med scand 184 1 1968

6 Braunschweig A Dunham L J & Nichols S Potassium and calcium content of gastric carcinoma Cancer Res 6 230 1946

7 DeLong R P Coman D R & Zeidman I The significance of low calcium and high potassium content in neoplastic tissue Cancer 3 718 1950

8 Dunham L J Nichols S & Braunschweig A Potassium and calcium content of carcinomas and papillomas of the colon Cancer Res 6 233 1946

9 Hahn Pedersen A Drewes V & Jesting E Gastric sediment fluorescence after administration of tetracycline Acta med scand 174 643 1963

10 Machado L Zaidman I Gerstein J F Lichtenberg F & Gray S J Factors affecting the site and degree of localization of tetracycline in sarcoma 37 tumors Cancer Res 24 1845 1964

11 Malek P Hammer J Zastava V & Pisa Z The diagnostic significance of fixation of tetracycline antibiotics in infarcted myocardium J cardiovasc Surg 61 827 1965

12 Rall D P Loo T L Lane M & Kelly M G Appearance and persistence of fluorescent material in tumour tissue after tetracycline administration J nat Cancer Inst 19 79 1957

13 Riley L H Tetracycline induced fluorescence in a transplanted human tumor Bull Johns Hopk Hosp 113 791 1963

14 Schlegel D & Uttermark D Krebsdiagnose durch zytologische Untersuchung in Verbindung mit dem Tetracyclin Fluoreszenztest Munch med Wschr 106 387 1964

15 Suntzeff V & Carruthers C Potassium and calcium in epidermal carcinogenesis induced by methylcholanthrene J biol Chem 153 521 1944

16 Tapp E Carroll R & Kovács K Tetracycline fluorescence in experimental renal lesions Arch Path 79 629 1965

17 Tapp E Kovács K & Carroll R Tetracycline staining of tissues in vitro Stain Technol 40 199 1965

18 Ullberg S Studies on the distribution and fate of S[35] labelled benzylpenicillin in the body Acta radiol (Stockholm) Suppl 118 1954
19 Vassar P S Saunders A M & Culling C. F A. Tetracycline fluorescence in malignant tumors and benign ulcers Arch Path 69 613 1960
20 Worsley G H McKenna R D & Beck I T Critical evaluation of the tetracycline fluorescence test in the diagnosis of gastric carcinoma Canad med Ass. J 88 1272, 1963
21 Yabe Y., Warren I A., Berk J E. & Kobernick S D Tetracycline fluorescence in mouse gastric carcinoma J Histochem Cytochem 12 84 1964

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BODY BUILD AND SERUM LIPIDS IN MALE PATIENTS HOSPITALIZED FOR PEPTIC ULCER OR MYOCARDIAL INFARCTION

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Abstract Two groups of patients have been studied One consisted of 63 peptic ulcer patients the other of 73 myocardial infarction patients All patients in both groups were treated in the Medical Clinic of the Danderyd Hospital during 1965 and were followed up about one year after discharge The infarction patients had significantly higher mean cholesterol and triglycerides than the ulcer patients (Table I) The difference occurred also between comparable age groups (Table II) The ulcer patients had a significantly higher skeletal quotient than the infarction patients implying that the former had a longer and more slender skeletal structure Comparison with the conscripts earlier described by Hellstrom (11) showed that in many respects the infarction patients resembled the short and heavily built conscripts whereas the ulcer patients resembled more the tall and lightly built

Several authors have shown that a relationship appears to exist between body build and serum lipids Gildea et al (9) in 1936 found higher cholesterol values in the serum of 17 men with pyknic body build selected according to Kretschmer s criterion than in 24 men with leptosome body build In 1954 Gertler et al (8) reported that the number of mesomorphs (heavy muscles and large bones) was larger in a group of 97 men suffering from coronary heart disease than in a control group of 146 healthy men Furthermore in the heart disease group the mesomorphs had a higher plasma cholesterol level than the remainder of the group In these studies the determination of body build was based either on a subjective evaluation or on a number of body dimensions which included complicated indices

Forssman et al (6) in 1958 reported on 66 male postcoronary patients whose body build was determined on the basis of length and size of skeleton They found that the patients with high cholesterol values also had large length and sturdiness factors of the skeleton

Bjurulf (2) in 1964 showed in a study of two postmortem materials that coronary atherosclerosis was more common in the short and muscular than in other men

In 1965 Hellstrom (11) reported that conscripts 19–20 years of age with short and thick skeleton had higher serum cholesterol than conscripts with long slender skeleton The heavily built conscripts also had a heredity of coronary heart disease which the tall and slender did not A heredity of peptic ulcer on the other hand was rather more frequent in the tall and slender than in the short and stocky The conscripts were grouped on the basis of body build determined according to body length and femoral condyle width as suggested by Lindegard (16) and also on the basis of the ratio of body length to femoral condyle width this ratio was earlier referred to by Hellstrom (10) as skeletal quotient According to this latter grouping procedure short and thickset persons have a low skeletal quotient and long and slender a high (11)

Earlier studies thus suggest that body build has a certain relation to the serum cholesterol level Except in the study by Hellstrom (11) however no account has been taken of the ratio of body length to femoral condyle width which should be a significant factor when deciding whether a person is heavily built or not For this reason the skeletal quotient has been determined for all patients in the present study in which a group of peptic ulcer patients is compared with a group which had had myocardial infarction The aim of the investigation was to study whether the find

ings in the conscripts (11) would in any way be repeated in a clinical material The special factors of interest were therefore the body build skeletal quotient and serum lipids of the ulcer and the infarction patients and their heredity of *peptic ulcer or myocardial infarction*

MATERIAL

The material consists of two groups of patients which are compared with one another One consists of 110 male patients hospitalized during 1965 in the Medical Clinic of Danderyd Hospital on a diagnosis of myocardial infarction In all cases the diagnosis was based on a typical clinical course verified by ECG and elevated GOT Of these 110 patients, 27 died during hospitaliza tion. Of the remainder two had had diabetes mellitus and were excluded from the survey Of the 81 patients discharged six died before the follow up could be made Finally there were two pat ents who for different reasons could not be persuaded to come for examina tion. Thus, of the 81 discharged patients 73 (90 I) were followed up The other material consists of 76 men who were admitted to the Medical Clinic during 1965 on a diagnosis of ventricular or duodenal ulcer The diag nosis was, in all cases confirmed radiologically either as a distinct niche or as a distinct deformation of the bu'b In the orig nal material six patients were found to have diabetes mellitus These cases have been excluded Since discharge from hospital five patients had d ed be fore the follow up could be made Furthermore two pa tients were lost trace of Of the original 76 cases there thus remain 63 (83) all of whom were followed-up It should be po nted out that all the patients were from *the reception area of the hospital this area which has* a populat on of rather more than 00 000 is in the northern part of the County of Stockholm, and does not cover the remainder of the county The two series were followed up about one year after discharge and have been earl er described in detail by Hellstrom (1, 13)

METHODS

In the follow up examination measurements were made of height, weight and blood pressure (diastolic pressure at the moment when the Korotkow sounds rapidly di minished in loudness) The patients ages were also re corded Measurements were made too of the skinfold thickness on the medioclavicular line at the le el of the navel and of the femoral condyle width in the right knee with the knee at an angle of 90 by the method reported earlier (15 16 17 18) The ratio of height to femoral condyle width has been referred to as the skeletal quo tient (10)

Samp es of venous blood were withdrawn at 8 a m. after an overnight fast Determinations were made of cholesterol and triglyceride le els in serum by the earlier described method (3 5) and also of the sedimentation rate and haemoglobin concentration As the triglycerides exhibited a skew distribution all statistical calculations have been made after logarithmic conversion of the value for the triglycerides as previously described (3 4)

Every patient was personally questioned by the examiner whether or not he kept any form of diet, whether he avoided milk butter and fatty food and whether he took nicotinic acid drugs The patient was also asked whether he had previously had a gastric ulcer or myo cardial infarction Moreover the patient was asked whether he considered he was suffering particularly from mental stress at the time of onset because of his work or for other reasons and if so whether the stress was so acute that it might be considered to have been a cause of his illness Finally all patients were asked whether either parent had been under medical care for gastric ulcer or myocardial infarction

RESULTS

Table I presents data on the 63 ulcer and 73 in farction patients in respect of age body dimen sions laboratory tests and blood pressures also the differences between the two groups The mean age of the infarction patients was found to be 11 6 years higher than that of the ulcer patients This difference was statistically significant As regards body build the data on height femoral condyle width and skeletal ratio for the infarc tion patients were for the most part nearly com parable with those for weight lifters and wrestlers reported on by Hellstrom (10) in 1961 The ulcer patients were taller and had a smaller femoral condyle width than the infarction patients but the differences were not significant In skeletal quotient however the ulcer patients had a sig nificantly higher value implying that they had a longer and more slender skeleton or less heavy skeletal build than the infarction patients The ulcer patients also had a significantly smaller skinfold thickness (Table I)

The ulcer patients had significantly lower sys tolic and diastolic blood pressures and cholesterol and triglyceride levels (Table I) The cholesterol and triglyceride levels for the ulcer patients were found to be largely similar to those of earlier described normal subjects of comparable age (1 3 4 14 19) whereas the infarction patients had higher values (cf 1 3 4) To further illustrat the difference a comparison has been made be tween the cholesterol and triglyceride levels of the ulcer and the infarction patients in compar able age groups The result is shown in Table II *The ulcer patients even in comparable age* groups were found throughout to have lower cholesterol and triglyceride levels than the infarc tion patients The material has also been grouped according to high and low cholesterol and tri

Table I *Differences between patients hospitalized for peptic ulcer and myocardial infarction*

	Patients with peptic ulcer				Patients with myocardial infarction					
	n	$\bar{X}$	S D	*e*	*n*	$\bar{X}$	S D	*e*	Diff	*P*
Age years	63	49 0	13 3	1 7	73	60 6	9 6	1 1	−11 6	
Height cm	63	175 1	7 7	1 0	73	173 3	6 2	0 7	+1 8	>0 1
Femoral condyle width cm	63	10 1	0 5	0 1	73	10 14	0 41	0 05	−0 04	>0 2
Skeletal quotient	63	17 4	0 8	0 1	73	17 0	0 7	0 1	+0 4	
Body weight kg	63	74 0	12 0	1 5	73	76 6	9 9	1 2	−2 6	>0 1
Skinfold thickness mm	63	17 6	8 1	1 0	73	23 8	7 0	0 8	−6 ..	
Systolic B P mm Hg	63	139 1	23 1	2 9	73	153 2	25 3	3 0	−14 1	
Diastolic B P mm Hg	63	89 2	12 1	1 5	73	97 1	13 8	1 6	−7 9	
Cholesterol mg/100 ml	63	249 0	50 0	7 0	73	285 6	53 6	6 3	−36 6	
Log triglycerides	63	1 94	0 30	0 03	73	2 18	0 24	0 03	−0 24	
ESR mm/h	63	8 8	10 2	1 3	73	14 8	18 6	2 2	−6 0	
Hb conc g/100 ml	63	14 7	1 2	0 1	73	14 6	1 2	0 1	+0 1	>0 2

Table II *Mean cholesterol and triglycerides of patients hospitalized for peptic ulcer and myocardial infarction by age groups*

	Patients with peptic ulcer			Patients with myocardial infarction		
Age (y)	*n*	Mean cholesterol (mg/100 ml)	Mean log triglycerides	*n*	Mean cholesterol (mg/100 ml)	Mean log triglycerides
0–35	9	231	1 93	—	—	—
36–45	18	258	1 96	5	292	2 14
46–55	16	245	1 96	20	297	2 24
56–65	14	248	1 91	23	293	2 14
66–83	6	252	1 89	25	268	2 18

glyceride levels as has been done in earlier investigations (7 14) Here it was found (Table III) that 48 (76 1 %) of the ulcer patients had normal cholesterol and normal triglycerides whereas the corresponding figures for the infarction patients were 30 (41 0 %) The mean value for the triglycerides measured in mg/100 ml was for the ulcer patients 98 6 (S D 53 5) and for the infarction patients 177 4 (S D 109 8) The high S D values indicate a skew distribution of the triglycerides Consequently all statistical calculations were made after logarithmic conver

Table III *High and low cholesterol and/or triglyceride levels in patients hospitalized for peptic ulcer and myocardial infarction*

		Patients with peptic ulcer				Patients with myocardial infarction			
		n	Age (y)	Mean cholesterol (mg/100 ml)	Mean log triglycerides	*n*	Age (y)	Mean cholesterol (mg/100 ml)	Mean log triglycerides
Cholesterol Triglycerides	<300 <150	48	47	234	1 87	30	62 2	250	1 99
Cholesterol Triglycerides	>300 <150	6	43	337	1 94	10	61 7	323	2 01
Cholesterol Triglycerides	<300 >150	7	54	260	2 31	15	60 6	258	2 38
Cholesterol Triglycerides	>300 >150	[illegible]	45	350	2 30	18	57 4	347	2 41

Table IV *Mental stress earlier peptic ulcer and earlier myocardial infarction in male patients hospitalized for peptic ulcer and myocardial infarction*

	Mental stress	Earlier peptic ulcer	Earlier myocardial infarction
Patients with peptic ulcer *n* = 63	41 (65 0%)	41 (65 0%)	0
Patients with myocardial infarction *n* = 73	42 (57 5%)	11 (15 1%)	11 (15 1)

sion of the value for the triglycerides as earlier *reported* (3 4)

Of the ulcer patients nine (14 1%) stated that they kept a moderate ulcer diet and three that they particularly avoided fats These patients did not materially differ from the others in blood fats body weight or skinfold thickness (12) *Similar results were obtained for the infarction* patients The 49 myocardial infarction patients who stated that they particularly avoided fats had as high blood fat values and skinfold thickness as the other 24 patients in the same group who kept no diet (13)

No significant correlation was found for either the ulcer or the infarction patients between cholesterol or triglycerides and age weight height femoral condyle width skeletal quotient or skinfold thickness

Mental stress (Table IV) was considered to have been present to such a degree that it contributed actively in bringing on the illness in 65 0% of *the ulcer and in 57 5° of the infarction pa*tients No less than 65 0% of the ulcer patients had earlier had a peptic ulcer but none a myocardial infarction Among the infarction patients (Table IV) 15 1% had had an earlier peptic ulcer and an equal number an earlier infarction

Table V *Patients reporting a history of myocardial infarction or gastric ulcer in a parent*

	Peptic ulcer	Myocardial infarction
Peptic ulcer patients *n* = 63	18 (28 5)	4 (6 3)
Myocardial infarction patients *n* = 73	6 (8 2)	11 (15 1)

One parent of 28 5% of the ulcer patients had had a peptic ulcer and one parent of 6 3% an infarction the corresponding figures for the infarction patients were 8 2 and 15 1% (Table V)

DISCUSSION

The 73 infarction patients had so short and heavy a skeletal structure that they were virtually comparable in this respect to weight lifters and wrestlers earlier reported on by Hellstrom (10) The 63 ulcer patients moreover had a significantly higher skeletal quotient than the infarction patients implying that they had a longer and narrower skeleton This difference in skeletal *structure must be regarded as due to differences* in the hereditary factors which determine skeletal build

The infarction patients had significantly higher mean cholesterol and triglyceride levels than the ulcer patients (Table I) Differences appeared also between comparable age groups (Table II) Thus some 34% of the ulcer and 59% of the infarction patients had elevated blood fats (Table III)

No less than 65 0 % of the ulcer and 57 5% of the infarction patients considered they had been in a state of mental stress which had actively contributed to their illness (Table IV) Thus mental stress seems to play an important role in the development of peptic ulcer and myocardial infarction Of the ulcer patients 65 0 had had a previous peptic ulcer but none a myocardial infarction Furthermore one parent of 28 5% of the ulcer patients had had a peptic ulcer and one parent of 6 3° a myocardial infarction Only 15 1% of the myocardial infarction patients had had a previous peptic ulcer and the same number a previous myocardial infarction 8 2° of the parents of these patients had had peptic ulcer and 15 1 myocardial infarction (Table V) These figures are perhaps evidence of an inherited tendency to react to mental stress in a particular way

The findings for the 63 ulcer and 73 infarction patients closely resemble those for the conscripts reported on by Hellstrom earlier (11) The infarction patients had a shorter and sturdier skeletal build and higher serum cholesterol level than the ulcer patients and a greater number of infarctions among the parents whereas a larger number of the parents of the taller and slimmer ulcer patients had suffered peptic ulcer

REFERENCES

1 Biorck, G Blomqvist G & Sievers J Cholesterol values in patients with myocardial infarction and in a normal control group Acta med scand 156 493 1957

2 Bjurulf P Atherosclerosis in different parts of the arterial system Amer Heart J 68 41 1964

3 Carlson L A Serum lipids in normal men Acta med scand 167 377 1960

4 — Serum lipids in men with myocardial infarction Acta med scand. 167 399 1960

5 — Determination of serum triglycerides J Atheroscler Res 3 334 1963

6 Forssman O & Lindegård B The postcoronary patient J psychosom Res 3 89 1958

7 Fredrickson D S & Lees R. S A system for phenotyping hyperlipoproteinemia Circulation 31 321 1965

8 Gertler M M & White P D Coronary heart disease in young adults Harvard Cambridge Mass 1954

9 Gildea E F Kahn E & Man E. B The relationship between body build and serum lip ds and a discussion of these qualit es as pyknophilic and leptophili factors in the structure of the personality Amer J Psychiat 9 1 47 1936

10 Hellstrom R Body build muscular strength and certain circulatory factors in military personnel Acta med scand Suppl 371 1961

11 — Body build and serum lipids Acta med scand 177 535 1965

12 — Serum lipids in male patients hospitalized for peptic ulcer Acta med scand 18? 699 1967

13 — Serum lipids in male patients hospitalized for myocardial infarction Acta med scand 182 727 1967

14 Jungner G & Jungner J Några resultat från den pågående Värmlandsundersokningen och några allmanna synpunkter på kemisk halsokontroll Svenska Lak Tidn 20 1710 1964

15 Kegs A & Brozek J Body fat in adult man Physiol Rev 33 245 1963

16 Lindegård B Variations in human body build Acta psychiat Suppl 86 1953

17 — Studies on the fat distribution in the adult male Lunds Universitets Årsskrift Lund 1956

18 — Differential somatology Lunds Universitets Årsskrift Lund 19?6

19 Tibblin G Aurell E Hjortzberg Nordlund H Paulin S Risholm L Sanne H Wilhelmsen L & Werko L A general health-examination of a random sample of ?0 year-old men in Goteborg. Acta med scand 177 739 1965

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THE NORMAL METABOLISM OF γG GLOBULIN

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Abstract A group of normal subjects has been studied after careful clinical examination Different methods of determining the intravascular γG globulin concentration and of calculating the catabolism were used The difference in the values as well as the composition of the material may well explain the divergent results published earlier A standardization of these methods is therefore proposed By using whole body counting as well we obtained further information which showed that the plasma and the whole body curves are parallel but not the plasma and the retained dose curves This must signify that if incomplete urine collection has been excluded ^{131}I is eliminated by routes other than the urine About 17 of the total daily amount of eliminated radioactivity can be estimated to be lost by routes other than the urine which in turn means that the catabolic value will be too low when calculated by the U/P method Possible routes of the ^{131}I-elimination would be perspiration and via the saliva Measurement of the extravascular pool by whole body counting showed that in healthy active subjects the extravascular compartment is equal in size to the intravascular

During the last five years the metabolism of γG globulin in control subjects has been studied by some authors (1 2 4 5 12) The results do not agree completely however Moreover some important questions are still unsolved So far it has not been established where the γG metabolism takes place or whether the activity excreted in the urine reflects the whole excretion nor is the true relationship between the extravascular and intravascular pools known

We present here a comparative study of the metabolism of γG globulin based on data obtained by whole body counting as well as by serum and urine analyses Such a study would provide further information that may explain the differences between the published metabolic values of control subjects and allow a comparison between values obtained by different methods of calculation and of measuring the serum protein

MATERIAL AND METHODS

The study comprised seven healthy volunteers (three men and four women) whose ages ranged between 17 and 24 years All had undergone careful medical examination including several laboratory tests None of them had any acute or chronic infections or other pathological processes which might influence the γG globulin situation and conventional blood and urine analyses were normal in all cases The relative electrophoretic serum protein distribution as well as the haematocrite values were normal throughout The results of these analyses were checked several times during the metabolic study

The subjects were investigated in the outpatient department and all carried on their ordinary work in the hospital during the period of study Three of the women menstruated once and one twice during the period of urine collection

Protein methods

G globulin was prepared from pooled normal sera by chromatography on DEAE Sephadex followed by gel filtration on Sephadex G 200 Phosphate buffer 0 05 *M* pH 8 0 was used

Labelling with I was done by McFarlane s method (6) The labelled protein preparation was tested for sterility and pyrogens

Quantitative determination of γG globulin in serum was done by (1) Determination of the gammaglobulin fraction by (*a*) paper electrophoresis (*b*) free boundary electrophoresis by the Tiselius method and (2) the single radial diffusion method described by Mancini et al (8) Total serum protein values were determined by a biuret method Comparisons were made on means of repeated samples

Performance of the isotope study

About 20 μCi ^{131}I labelled γG globulin (equalling at the most a few mg of γG globulin) was injected intravenously Blood samples were taken ten minutes after the injection and then daily for two weeks thereafter every second to third day The plasma volume was calculated by the isotope-dilution technique The subjects were carefully instructed to save all the urine for each 24-hour period From these amounts samples were taken for measurements of radioactivity Usually the urinary activity could not be measured with satisfactory precision after 25 days

Whole body counting was done one and three hours after the injection of the isotope and then simultaneously with the blood samples

In all cases sodium iodide solution (ten drops of 10% NaI daily) was given three days before and throughout the period of investigation which covered 50 days

Methods for calculation of metabolic data

Mathematical analysis of the plasma activity curve was done by Matthews method (9) In addition the 24-hour urinary excretion of activity (U) was divided by the mean activity in plasma during 24 hours (P) The resulting ratio U/P is a measure of the catabolism and is constant throughout the period of study if the breakdown occurs in the intravascular pool or in a compartment with rapid exchange with the intravascular space

The excretion of activity was obtained also by the differences between successive whole-body counts The ratio of the difference between two successive whole body counts to the plasma activity can be used to obtain a measure of the catabolism

The calculations were made with a digital computer with two ALGOL programs PLASMA I and PLASMA II (10) All U/P and whole body calculations were performed on measurements between days 5 and 26

Methods for determination of whole body activity

The patient lies supine on a stretcher in a steel walled room One scintillation detector with a sodium iodide crystal moves above and another below the stretcher All pulses over 0 1 Mev were counted in order to obtain at sensitivity The patient s background activity was ...red before injection of isotope The efficiency of measurement of I was about 1% Further details about the whole body counter are published elsewhere (11)

Measurement of radioactivity

Four ml of plasma or urine were measured in a well scintillation counter Only the activity in the photopeak at 0 36 Mev for ^{131}I was recorded The activity in menstrual fluid was analysed by measuring the sanitary towels in a special liquid scintillation counter (Packard Armac extra large)

Definitions

Catabolism The normal or pathological process by which the molecules of a substance are converted to other molecules or ions

Fractional catabolic rate Catabolism per unit of time expressed as a percentage of the intravascular pool If the size of this pool is known the catabolism can be expressed in g per day

Retained dose The body s total activity calculated by subtracting the total urinary and faecal excretion of activity from the given dose

Whole body activity The body s total activity measured with a whole body counter

Pool The area or the form in which a substance occurs A pool may be a physiologically or chemically defined form The feasibility to define a pool is dependent on its rate of exchange with other pools in relation to sampling intervals and the time of investigation

RESULTS

Table I shows the total serum protein value and the γ globulin concentration in serum determined by paper electrophoresis free boundary electrophoresis and immunologic analysis The plasma volume was also recorded as was the intravascular content of γ globulin which was calculated by the three methods The γ globulin values obtained by paper electrophoresis were highest while free electrophoresis and immunologic analysis of γG globulin gave virtually identical mean values The γ globulin values obtained by paper electrophoresis are 8–10% higher than the other values The difference between this

Table I *Gamma G globulin concentration and intravascular (IV) gamma-G globulin content according to different methods in seven normal subjects*

Case no	Total protein (g/100 ml)	Gamma G globulin (g/100 ml)			Plasma vol (l)	IV gamma-G globulin (g)		
		Electrophoresis				Electrophoresis		
		Paper	Free boundary	Mancini method		Paper	Free boundary	Mancini method
1	8 2	1 29	0 98	1 24	3 10	40 0	30 4	38 4
2	7 9	1 08	0 93	0 90	2 82	30 5	26 2	25 3
3	7 7	1 04	0 92	0 98	3 01	31 3	27 7	29 3
4	7 9	0 91	1 23	0 83	3 00	27 3	36 9	24 8
5	7 5	1 07	1 15	1 07	3 62	38 7	41 6	36 8
6	7 9	1 18	1 08	0 98	1 98	23 4	21 4	19 4
7	8 1	1 05	0 75	0 98	2 67	28 0	20 0	26 1
Mean	7 9	1 09	1 01	0 99	2 89	31 3	29 2	28 6
S D	0 2	0 12	0 16	0 13	0 50	6 1	7 9	6 8

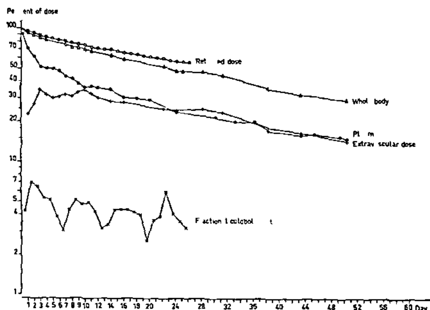


Fig 1 The catabolism of γG globulin in a normal subject (The fractional catabolic rate is expressed in of intravascular pool per 24 h)

method and the immunologic method in this respect is significant ($p < 0.01$) The difference between the values obtained by the different quantitation methods are of course reflected in the figures obtained from calculation of the intravascular γG globulin concentration

Fig 1 shows the results from a study of one of the subjects In this as in the rest of the investigated cases there is a constant catabolism which indicates that non denatured protein preparations were used Neither in this nor in the other cases were the curves for retained dose and plasma activity parallel The whole body analyses showed in all cases a minor but unmistakable discrepancy between the whole body and the retained dose curves The plasma curve extravascular curve and the whole body curve on the other hand run parallel throughout the latter part of the analyses

As will be s en from Table II the catabolism was calculated by three different methods The fractional catabolic rate obtained by mathematical analysis was virtually identical to that calculated from whole body data The U/P analysis which is based on urine measurements on the other hand gave lower values with significant deviation

Table II *Catabolism and extravascular/intravascular distribution (EV/IV) of gamma-G globulin according to different methods in seven normal subjects*

Case no	Catabol sm									EV/IV	
	U/P	Matthews	Whole body	U/P	Matthews	Whole body	U/P	Matthews	Whole body	Whole body	Matthews
	(/day)			(g/24 h)			(mg/24 h/kg)			(day 23 27)	
1	4 8	5 7	5 7	1 84	2 19	2 18	27	32	32	0 64	0 83
2	4 6	5 9	5 4	1 17	1 49	1 37	17	21	20	1 07	1 13
3	3 9	5 4	5 0	1 13	1 58	1 46	20	29	26	1 02	0 96
4	4 2	4 7	5 0	1 03	1 17	1 24	12	14	15	0 97	0 87
5	4 8	6 5	6 4	1 75	2 39	2 37	18	25	24	1 07	0 83
6	3 7	4 8	4 8	0 72	0 93	0 93	15	19	19	1 05	0 99
7	6 6	7 4	7 6	1 73	1 93	1 99	22	25	26	1 07	1 17
Mean	4 65	5 77	5 70	1 34	1 67	1 65	19	23	23	0 98	0 97
S D	0 97	0 95	1 01	0 43	0 53	0 53	5 0	6 1	5 7	0 15	0 14

Table III *Per cent of injected dose distributed intra (IV) and extravascularly (EV) excreted and retained in the body according to two calculation methods in seven normal subjects*

	IV	Retained dose	Total urinary excretion	EV from retained dose	Whole body activity	EV from whole body activity	Difference between retained dose and whole body activity
Day 5							
Mean	46 0	79 7	20 3	33 7	76 6	30 6	3 1
S D	3 89	6 21	3 84	2 61	4 46	2 96	0 89
Day 25							
Mean	21 2	52 1	47 9	30 8	42 2	21 0	9 8
S D	3 06	6 41	6 30	4 15	6 46	4 20	1 47
Day 38							
Mean	15 2	—	—	—	31 1	15 9	—
S D	3 0?				5 99	3 72	

in comparison with both the whole body method and with Matthews method ($p < 0.001$) This of course influences the metabolic figures expressed both as g per 24 hours and as mg per 24 hours per kg

Table III shows the intravascular and extra vascular distribution calculated from retained dose and whole body activity 5 25 and 38 days after the start of the analyses The table does not record any retained-dose data at 38 days as the elimination via the urine could not be deter mined with sufficient accuracy at this time

After five days there was still much more γG globulin intravascularly than extravascularly A difference of 3 1 % of the given dose was noted between the activity remaining in the body cal culated as retained dose and estimated as whole body activity After 25 days there was as much γG globulin intravascularly as extravascularly if the calculation is based on whole body data or mathematical analysis The extravascular distribu tion and activity remaining in the body calculated as retained dose showed values that were 9 8 % higher than those determined by whole body anal ysis which can be interpreted as an indication that this activity left the body by routes other than the urine

DISCUSSION

The results of five earlier studies with non dena tured γG globulin and the results of the investiga tion presented here are compiled in Table IV

It will be seen from Table IV that divergent results have been obtained for catabolism ex pressed in per cent per day as well as in g per

Table IV *Catabolism of gamma-G globulin according to different authors*

Authors	No of cases	Type of subjects	Catabolism (/24 h) Methods of calculation[a] A	B	C	D	E	(g/24 h)	(mg/24 h/kg body weight)
Cohen & Freeman (5)	5	Normals	5 2	5 8	5 1		3 0	2 1[b] 2 4 (E)	
Birke et al (4)	10	Controls			4 8			1 7 (C)	25
Solomon et al (12)	14	Controls					3 0	2 3 (E)	36
Ahlinder et al (1)	15	Controls			4 8			1 8 (C)	25
Andersen (3)	21	Convalescents			6 9			2 5 (C)	36
Present invest	7	Normals		5 8	4 7	5 7		1 7 (B) 1 3 (C) 1 7 (D)	23 19 23

[a] A = equilibrium time B – Matthews C = U/P D = Whole body counting
E = From $T\frac{1}{2}$ of plasma activity curve and total body gamma G globulin pool
[b] Calculated from mean of methods A B and C

day and mg p r kg per day These discrepancies might have several causes which are worth discussing Catabolism expressed in g per 24 hours or in mg per kg per 24 hours depends partly on the method used for determining the γG globulin concentration in serum Therefore we determined the γG globulin concentration by three different methods Paper electrophoresis yields values that are 8–10 higher than those obtained by the other two methods The difference in this respect between paper electrophoresis and immunologic analysis is significant whereas free boundary electrophoresis gives numerically lower values without statistical significance in comparison with the values of paper electrophoresis The difference between paper electrophoresis and the other two methods may be expected to be higher and assume still greater importance in studies of patients with dys γ globulinaemia especially those with hypo- or a γ globulinaemia

The free boundary electrophoresis and the immunologic methods gave virtually the same mean values in this study In our previous analyses we used free electrophoresis but in view of the similar results obtained by the immunologic method and as this analysis is fairly simple to perform and does not require expensive equipment we employed it in the present study and shall continue to do so

The second factor that influences the results is the choice of method for calculation of the catabolism expressed in per cent per day The urine/plasma (U/P) analysis gives a value of 4 7° which is in agreement with that previously reported by us in control subjects of varying ages but slightly lower than Cohen and Freeman s data (5) and distinctly lower than those of Andersen s control cases (3) All three authors use the same method of calculation Solomon et al — and also Cohen and Freeman—on the other hand calculate the catabolism from the slope of the plasma disappearance curve and the total gamma globulin pool Their result 3 ₀ per day is slightly higher than the average of the other methods if an equal distribution of the gamma globulin between intra and extravascular pools is assumed The mathematical and the whole body analysis both give catabolic rates of about 5 7 per day which is about 21 higher than the value obtained by the U/P method This difference is significant The causes and the importance of this difference will be discussed later on but it is worth mentioning that whole body counting and Matthews method give similar values Thus the method of calculating the catabolism and of determining the serum γG globulin concentration may explain some of the varying results reported for control cases

The lack of parallelism between the plasma curves and the retained-dose curves which was noted in these and in previously reported control cases should be discussed as its interpretation may be thought to influence the results

The whole body count was made on the whole gamma spectrum and not on the photopeak alone Thus redistribution errors were minimized Control measurem nts have indicated that these errors were well below one per cent (11) Therefore it does not seem probable that the technical performance of whole body measurement influenced the values obtained

Accumulation of ^{131}I in the body as iodide is improbable The thyroid uptake was blocked by inert iodine in this and in other investigations Studies of larger control series have shown that under this condition < 1 ₀ is taken up by the thyroid Cohen and Freeman studied the elimination of ^{131}I and showed that it is neither taken up by nor fixed in any other tissue of the body (5) This was confirmed by whole body counting in one of our cases given ^{131}I (2) Our studies with ^{131}I tyrosine (monoiodotyrosine) show some delay of elimination but no accumulation (2) As there is thus no accumulation of ^{131}I metabolites in control cases there may be three alternative explanations of the lack of parallelism between the retained-dose and the plasma curves 1 incomplete collection of urine 2 losses of ^{131}I by routes other than the urine or 3 a globulin pool that is slowly exchangeable in relation to the period of investigation

It is evident from the data that the difference between the retained-dose and the whole body curves increases each day by a certain amount and reaches a m an of 9 8° of the given dose after 25 days This means that about 17% of the total daily loss of activity occurs by routes other than the urine The whole body and the plasma curves are parallel which signifies that when urinary collection is incomplete in these as in the previously reported cases exactly the same proportion of the 24 hour urine is lost each day

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THE TUBULAR REABSORPTION OF CALCIUM IN PRIMARY HYPERPARATHYRO IDISM AND IN NON PARATHYROID HYPERCALCEMIA[1]

Ib Transbøl Steffen Hahnemann and Ib Hornum

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Abst act The tubular reabsorption of calcium, expressed in percentage of the filtered load of calcium (TRCa) and the phosphate excretion index were examined in 31 hypercalcemic patients to evaluate their relati e value in the differential diagnosis of hypercalcemia The phos phate excretion index proved to be of no value

High TRCa% values were found in primary hyper parathyroidism (15 pat) in hyp rparathyroidism and sarcoidosis (1 pat) and in hyp rcalcemia attributed to metabolic alkalosis (? pat.) The elevated TRCa values in the last named condition are probably explained by a specific effect of the metabolic disturbance on the renal tubule It is inferred that an increased tubular reabsorp tion of calcium may contribute to the development and maintenance of the hypercalcemia in the milk alkali syndrome

Low TRCa % values were found in poststrumectomy hypoparathyroidism overdosed with vitamin D (3 pat) ord nary hypercalcemic sarcoidosis (5 pat) idiopathic intest nal hyperabsorpt on hypercalcem a (1 pat) Hodg kin s paragranuloma (1 pat) myelomatosis (1 pat) and surprisingly in hyperparathyroid sm associated w th sar coidosis (1 pat.) and with id opathic hypercalcuria (1 pat)

It is concluded that TRCa%—with only a few excep tions—reflects the functional state of the parathyroid glands in hypercalcemia being *elevated* in the presence of hypersecretion of parathyroid hormone and *depressed* in non parathyroid hypercalcemia in which functional hypoparathyroidism is assumed to be pre ent.

In primary hyperparathyroidism the 24-hour renal excretion of calcium is of minor diagnostic im portance since normal excretion as well as raised excretion are frequently found (38 56) The urinary calcium excretion depends on glomerular filtration and tubular reabsorption and is thu in fluenced by 1 the concentration of ultrafiltrable calcium in plasma 2 the glomerular filtration rate 3 factors affecting the capacity of tubular reabsorption The ultrafiltrable fraction (UFCa) of the total serum calcium comprises a large free or ionized part (Ca^{++}) and a small complex bound one of which the latter is less completely reabsorbed (3 34 59) However the complex bound fraction undergoes unpredictable changes in the tubules and therefore it is only possible to determine the net reabsorption of the total ultrafiltrable fraction The reabsorption comprises from 94 to over 99% of the filtered calcium (14 36 38 40 48) it is active (14 42) and takes place along the entire nephron (34 42) About 70% of the reabsorption takes place in the proximal tubules 20–25 % in Henle s loop and 10 in the distal tubules and the collecting ducts (42) An increase in the filtered load of calcium raises the absolute amounts of calcium reabsorbed (14 36 46) but the reabsorption *in percentage* of the filtered load decreases (38 46) No T_m value for the reabsorption of calcium can be demon strated (14 46) The calcium reabsorption in the proximal tubules is enhanced by the proximal reabsorption of sodium and is therefore in fluenced by variations in this parameter (18 39 59)

The parathyroid hormone increases the tubular reabsorption of calcium as is demonstrated in rats (53 54) in dogs (60) and in man (4 38) The parathyroid hormone seems to increase the reab sorption in the distal tubules only (18 60) The effect of thyrocalcitonin on the tubular reabsorp tion of calcium is still unknown

Against the background of the well-established feed back interplay between Ca and the para

[1] Presented in part at the IVth International Sarcoidosis Conference September 1966 Paris and at the VIth Acta Endocrinolog ca Congress Au ust 1967 Helsinki

Table I *Summary of the dietary intake of calcium and phosphorus and those serum and urine parameters essential for the calculation and interpretation of the tubular reabsorption of calcium (TRCa %) and the phosphate excretion index (P E I) (Thirty-one patients with hypercalcemia and four normal subjects)*

Pat no	Initials	Sex	Age	Diet: Type	Diet: Ca cont (mg/24 h)	Diet: P cont (mg/24 h)	Serum: P	Serum: Total Ca (mg/100 ml)	Serum: Ca (mg/100 ml)	Serum: UFCa	Serum: Stand bic (mEq/l)	Serum: pH capill blood	Urine: CCr (ml/min)	Urine: Ca (mg/24 h)	Urine: TRCa (%)	Urine: 24 h P E I	Urine: Na (mEq/24 h)
Normal range							2.3–4.6	9.20–10.60	6.00–6.60	6.55–7.63	21.3–25.8	7.36–7.42				−0.08–+0.09	
Primary hyperparathyroidism																	
1	BSH	♀	24	C	—	—	3.0	11.00	7.25	8.50	22.4	7.42	83.0	153	98.5	0.00	—
2	JAN	♂	24	B	941	1180	2.8	11.17	7.55	8.50	24.0	7.41	89.9	160	98.6	+0.20	45
3	IT	♂	53	C	—	—	1.7	12.80	8.60	9.80	[illegible]	7.41	81.7	208	98.2	+0.31	—
4	JM	♂	29	B	864	1200	2.7	11.55	7.95	8.75	23.2	7.41	152.4	574	97.3	+0.10	105
5	GMP	♀	57	C	—	—	3.5	14.70	9.90	11.45	23.7	7.45	18.0	118	96.0	+0.48	—
6	AHJ	♀	48	B	917	1055	2.6	17.15	11.85	13.03	25.4	7.47	42.5	363	95.5	+0.30	45
7	NKC	♀	62	B	724	1121	2.9	11.00	7.45	8.35	23.8	7.39	82.8	162	98.4	+0.09	60
8	AHO	♂	52	B	810	—	2.2	14.23	10.13	11.00	24.0	7.43	82.0	472	96.4	+0.33	54
9	EKJ	♀	56	C	—	—	2.4	11.60	7.85	8.80	22.5	7.42	70.7	105	98.8	+0.20	—
10	KJ	♂	55	B	705	1173	3.4	11.40	7.55	8.60	23.0	7.40	100.2	366	97.1	+0.09	—
11	MH	♂	48	C	—	—	2.9	11.13	6.98	7.88	24.8	7.39	150.8	488	97.2	+0.10	—
12	EE	♀	58	C	—	—	2.8	10.60	6.70	7.55	24.0	7.41	85.3	190	98.0	+0.07	—
13	CWJ	♂	51	A	793	1070	3.1	15.02	9.93	11.36	23.0	7.42	38.4	149	97.6	+0.37	57
14	EJP	♀	41	A	804	1051	2.0	14.80	10.75	11.93	22.6	7.35	78.6	337	97.5	+0.36	72
15	VPS	♂	45	C	—	—	2.9	10.15	6.75	7.65	23.9	7.40	108.4	565	95.3	+0.12	—
16	HH	♂	72	C	—	—	3.3	9.83	6.68	7.52	23.3	7.18	50.8	123	97.7	+0.14	—
Hyperparathyroidism associated with sarcoidosis																	
17	MES	♀	67	B	901	1156	3.0	12.00	8.35	9.50	22.5	7.39	61.9	178	97.9	+0.70	63
18	BES	♀	23	C	—	—	2.7	11.80	8.05	9.15	23.9	7.46	45.1	386	93.5	+0.42	11
—	—	—	—	A	870	1096	2.7	12.64	8.54	9.93	27.1	7.48	49.3	356	94.9	+0.38	72
Ordinary hypercalcemic sarcoidosis																	
19	EVK	♀	58	C	—	—	3.3	13.00	8.25	10.05	23.6	7.39	13.2	779	83.7	+0.53	104
—	—	—	—	A	839	1109	3.5	11.19	7.61	8.90	23.2	7.39	13.7	594	86.1	+0.45	77
20	ABH	♂	69	C	—	—	3.7	14.50	—	11.42[a]	—	—	29.7	781	84.0	+0.38	—
21	KMC	♂	33	C	Anorexia Vomiting		4.4	13.63	9.55	10.88	26.4	7.43	32.9	317	90.1	+0.27	61
22	LKK	♂	25	C	—	—	2.6	10.90	7.10	8.90	24.1	7.43	109.3	562	96.0	—	—
—	—	—	—	A	1117	1179	3.1	11.78	8.04	9.49	24.3	7.42	84.5	339	95.3	+0.13	—
23	TFS	♂	6	C	—	—	4.2	11.25	7.65	8.45	25.1	7.43	48.9	(22 h) 544	90.0	+0.21	—
—	—	—	—	B	705	1173	4.5	10.63	7.25	8.18	27.7	7.45	72.8	159	95.8	+0.06	33
—	—	—	—	A	720	1122	4.5	10.97	7.23	8.47	25.1	7.42	68.7	376	95.5	+0.04	61

	PEO	♂	5	A	821	1114	4 2	11 56	7 60	8 85	28 9	7 47	23 6	361	88 0	+0 47	—
5	EB	♀	1	B	778	1156	3 0	11 8	7 13	8 28	24 3	7 47	37 0	387	91 3	+0 37	72
26	MKH	♀	66	C	—	—	3 0	11 60	8 05	9 15	—	—	28 7	456	88 0	+0 42	—
Idiopathic intestinal hyperabsorption hypercalcemia																	
27	HOCN	♂	44	C	—	—	3 2	10 80	7 08	7 97	25 0	7 41	117 6	732	94 4	+0 08	—
—	—	—	—	A	866	1154	3 6	10 60	7 27	8 01	3 9	7 38	114 7	616	95 3	+0 01	55
Hodgkin s paragranuloma																	
28	BSH	♂	28	C	—	—	3 6	9 90	6 60	7 50	25 6	7 47	75 3	371	95 5	+0 08	122
—	—	—	—	C	—	—	3 9	13 40	7 93	9 45	25 5	7 41	23 3	365	88 5	+0 36	110
Myelomatosis																	
9	VO	♂	67	C	—	—	4 0	11 90	8 90	10 10	25 5	7 43	52 8	358	95 3	+0 11	—
Hypercalcemia associated with metabolic alkalosis and hypokalemia																	
30	SHC	♀	39	A	1177	1314	4 2	10 33	6 90	8 43	32 1	7 46	76 6	56	99 4	−0 03	77
					(Vomiting)												
31	ECMH	♀	44	A	1240	1357	2 8	11 40	7 35	8 85	28 3	7 49	74 6	73	99 2	+0 16	129
Normal subjects																	
32	AM		20	A	749	1107	4 2	10 00	6 48	7 45	24 0	7 41	107 4	187	98 4	−0 06	169
33	JG	♂	23	A	807	1163	4 0	9 85	6 85	7 50	24 5	7 41	113 9	00	98 4	−0 03	79
34	SM	♂	23	A	807	1163	3 7	9 90	6 38	7 40	24 5	7 40	120 4	214	98 3	−0 01	72
35	LH	♂	4	A	807	1163	3 9	9 60	—	7 18	23 8	7 40	115 6	243	98 0	+0 02	69

UFCa was calculated from the total calcium concentration by use of the mean total calcium/UFCa ratio in ordinary hypercalcemic sarcoidosis

thyroid hormone secretion (51) it may be surmized that hypercalcemic subjects have either increased or decreased secretion of parathyroid hormone The secretion will be raised in primary hyperparathyroidism (5) and in malignant tumors producing parathyroid hormone like substances (55) while a decreased secretion must be expected in hypercalcemia of non parathyroid origin As the parathyroid hormone increases the tubular reabsorption of calcium it may thus be anticipated that raised tubular reabsorption is found in hypercalcemia owing to primary hyperparathyroidism and reduced tubular reabsorption in hypercalcemia of non parathyroid origin Preliminary observations have been in accordance with this hypothesis (57) The present publication represents a further testing of the hypothesis

MATERIAL

The study comprises thirty-one hypercalcemic patients and four normal subjects (Table I) Hypercalcemia is here defined by the presence of an increased concentration of Ca^{++} There were the following causes for hypercalcemia primary hyperparathyroidism (16 pat) hyperparathyroidism associated with sarcoidosis (2 pat) ordinary hypercalcemic sarcoidosis (5 pat) post trumectomy hypoparathyroidism overdosed by vitamin D (3 pat) idiopathic intestinal hyperabsorption hypercalcemia (1 pat) hypercalcemia due to Hodgkin s paragranuloma (1 pat) myelomatosis (1 pat) and hypercalcemia associated with metabolic alkalosis and hypokalemia (pat) The latter condition was due to vomiting in anorexia nervosa (case no 30) and to overtreatment with sodium bicarbonate in renal tubular acidosis (case no 31) The diagnosis of primary hyperparathyroidism was verified histologically by parathyroidectomy (cases no 1–13 17–18) and by a biopsypro en generalized osteitis fibrosa (case no 14) Finally in ases no 15–16 a histologically negative neck exploration was followed by a lasting normalization of Ca^{++} The diagnosis of sarcoidosis Hodgkin s paragranuloma and myelomatosis was confirmed histologically Further information, concerning the patients with idiopathic intestinal hyperabsorption hypercalcemia, with the combination of hyperparathyrodism and sarcoidosis and with some of the other conditions, will be found in earlier publications (4 5 3, 33 57 58) The initials of the patients can be used for identification Separate papers will appear on the serum calcium fractions in hypercalcemia and on idiopathic intestinal hyperabsorption hypercalcemia, ordinary hypercalcemic sarcoidosis Hodgkin s paragranuloma hyperparathyroidism associated with sarcoidosis and metabolic alkalosis

METHODS

Attempts were made to perform the examinations under normal intake of calcium, phosphorus sodium and protein. In the majority of the patients examined the

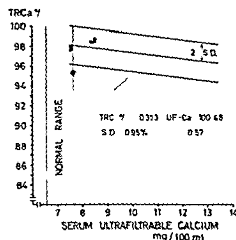


Fig 4 The tubular reabsorption of calcium (TRCa %) related to the concentration of ultrafiltrable calcium in serum Sixteen patients with primary hyperparathyroidism (●) and two patients with sarcoidosis *and* hyperparathyroidism (×) The patient with primary hyperparathyroidism *and* idiopathic hypercalcuria is indicated by a circle (see text)

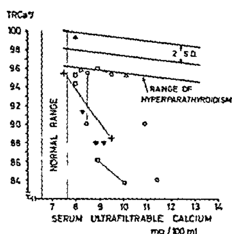


Fig 5 The tubular reabsorption of calcium (TRCa %) related to the concentration of ultrafiltrable calcium in serum Thirteen patients with non parathyroid hypercalcemia The following disorders are represented ordinary hypercalcemic sarcoidosis (O) poststrumectomy hypoparathyroidism overtreated by vitamin D (▽) idiopathic intestinal hyperabsorption hypercalcemia (□) myelomatosis (Δ) Hodgkin's paragranuloma (+) and metabolic alkalosis and hypokalemia (▲)

primary hyperparathyroidism Some of the patients were examined at different levels of UFCa or on several admissions which is indicated by a line between the TRCa % values in question Generally the TRCa % tended to be lower at the higher concentrations of UFCa but great individual variations are seen

The Phosphate Excretion Index (P E I)

P E I values above +0 09 were found in twelve out of sixteen patients with primary hyperparathyroidism but also in several patients in the group of non parathyroid hypercalcemia Some patients were examined at different CCr levels or on various admissions which is indicated by a line between the P E I values in question In the cases where CCr was below 55–65 ml/min P E I was found to be inevitably raised *irrespective of the cause of the hypercalcemia* Two patients (cases nos 23 and 28) with non parathyroid hypercalcemia had normal P E I at CCr above 70 ml/min but raised P E I at CCr below 50 ml/min (Fig 6)

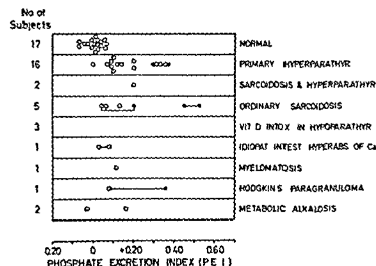


Fig 6 The 24 hour phosphate excretion index (P E I) Thirty one patients with hypercalcemia and seventeen normal subjects The dashed lines represent the normal range (mean ± 2 s D) It is indicated if the 24-hour clearance of creatinine is above (O) or below (●) 50 ml per minute

DISCUSSION

The Interpretation of TRCa

It must be emphasized that the TRCa % values from different laboratories are not comparable The results depend upon whether serum or plasma is used which part of the day the examination covers the intake of calcium and phosphorus (38) as well as the sodium intake (39) and the degree of renal insufficiency in the subjects studied (7)

Serum versus plasma

It has been shown by Hahnemann (23) that heparin is a calcium complexing agent Accordingly serum UFCa is higher than plasma UFCa This fact may explain why the 24 hour TRCa % values in our four normals 98 0–98 4% are slightly higher than in those of Kleeman et al (38)

Duration of the study

Laake (40) and Kleeman et al (38) found great variability in the TRCa % in normal subjects (92–99 5%) in clearance experiments of short duration while the variability was considerably less in 24 hour studies (38) In clearance experiments of short duration Kleeman et al (38) also found a remarkably lower TRCa % in primary hyperparathyroidism than has been found by any other investigator (11 21 22 40 41) An explanation for these differences is not immediately apparent The most essential theoretical objection to the 24 hour TRCa % is that we do not know whether the used UFCa value is representative of the 24 hours Carruthers et al found no diurnal variation in serum non protein bound calcium (13) while the observations regarding serum total calcium are contradictory (9 13) Despite the objection mentioned above the great variability of the TRCa % in short term studies made the 24 hour determinations preferable

Serum ultrafiltrable calcium

UFCa is essential both for the calculation and for the interpretation of TRCa % (38) When we use TRCa % instead of TRCa expressed in mg per minute we have corrected for one of the two parameters which determine the filtered load of calcium namely the glomerular filtration rate Therefore the second parameter UFCa is the sole indicator of the filtered load of calcium Investigators who did not interpret the TRCa % in relation to UFCa (as exemplified in Fig 2) arrived erroneously at the conclusion that PTH either does not affect or may even reduce the TRCa % (11 21 22 40) The demand for comparability with regard to the UFCa implies that the TRCa % in hypercalcemic patients *cannot* be compared with the TRCa % in normal subjects

The level of parathyroid activity

The effect of parathyroid hormone on TRCa % as it can be estimated from the observation of the TRCa % in primary hyperparathyroidism and in hypoparathyroidism has been the subject of much discussion The most controversial finding has been a very high TRCa % in untreated hypoparathyroidism (21 22) The probable explanation is that parathyroid hormone only affects the reabsorption of calcium in the distal tubules By sufficiently low filtered loads of calcium practically all filtered calcium is reabsorbed in the proximal tubules and the reduced parathyroid hormone secretion cannot manifest itself Kleeman et al (38) contributed essentially to our knowledge of the effect of parathyroid hormone on TRCa % in man Their most important findings were 1 that an increase in the filtered load of calcium decreased the TRCa % in all subjects and 2 that for any given filtered load of calcium the greater the level of parathyroid activity the higher the TRCa %

The glomerular filtration rate

At clearance levels below 50 to 60 ml per minute the TRCa % decreases sharply in the group of non parathyroid hypercalcemia this was not the case in primary hyperparathyroidism (Fig 3) Our studies offer no explanation of this obvious difference Better et al found that the TRCa % decreased with decreasing glomerular filtration rate in uremia an observation which they attributed to a raised filtered load of sodium *per nephron* (7 8) The different effect of decreasing glomerular filtration rate on the TRCa % found in our two groups of hypercalcemias is however hard to explain in this way especially as the few available data on the renal sodium excretion showed no decisive difference between the two groups (Table I) It is important however to realize that the decreasing TRCa % represents a

way of escape from the rise in serum calcium concentration which would be a consequence of a lowered glomerular filtration rate this might be mediated by a suppression of the parathyroid glands The lack of escape in primary hyperparathyroidism may be due to the autonomous non suppressible function of the parathyroid adenomas or primary hyperplasias

A similar escape phenomenon with decreasing glomerular filtration rate is seen for *phosphorus* But in contrast to the TRCa % the TRP % decreases (P E I increases) equally at low clearance levels in the two groups of hypercalcemias (see later) If the explanation of the escape phenomenon for calcium is true the escape phenomenon for phosphorus does *not* seem to be mediated through the parathyroid glands

Hypercalcemic States Associated with Elevated Levels of TRCa %

Primary hyperparathyroidism

The normal 24-hour urinary calcium excretion in many of our patients corresponds to the findings of others (38 56) It must be stressed that a normal urinary calcium excretion in a hypercalcemic patient must never be used as an argument against the diagnosis primary hyperparathyroidism—on the contrary! The high TRCa values and the decreasing tendency of TRCa with increasing UFCa (Fig 4) agree with the conclusions of Kleeman et al (38) Case no 15 whose TRCa % was remarkably low 95 3% remained hypercalcuric (381 mg/24 h) during a normal calcium intake postoperatively although Ca^{++} was normalized He is supposed to have had idiopathic hypercalcuria as well as primary hyperparathyroidism Similar patients have been described previously (17 22 p 313)

Hyperparathyroidism associated with sarcoidosis

Case no 17 is characterized as hyperparathyroid by presenting cortisone resistant hypercalcemia parathyroid adenoma(s?) and an increased maximal tubular reabsorptive capacity for glucose (TmG/GFR) (25 58) In good agreement with this the TRCa % was within the range of primary hyperparathyroidism (Fig 4) In case no 18 the assumption of hypersecretion of parathyroid hormone was supported by insufficient suppression of Ca^{++} during cortisone treatment chief cell hyperplasia of three parathyroid glands and an increased TmG/GFR After parathyroidectomy Ca^{++} TmG/GFR TRCa % and P.E.I were normalized (58) In spite of the evidence cited for parathyroid hyperfunction the preoperative TRCa % values were definitely reduced Our present knowledge does not enable us to explain this finding

Hypercalcemia associated with metabolic alkalosis and hypokalemia

The combination of hypercalcemia and normocalcuria is frequently found in the so-called milk alkali syndrome (10) A raised calcium intake is usually considered to be mainly accountable for the hypercalcemia but instances where the syndrome developed during high alkali—normal calcium—intake have been described (for review Punsar and Somer (47)) Although marked hypercalcuria may occur in patients with considerably reduced CCr caused by ordinary hypercalcemic sarcoidosis (Table I cases no 19 and 20) the normocalcuria in the milk alkali syndrome is generally attributed to renal insufficiency Ingestion of alkali reduces the urinary calcium excretion in normal subjects (15) and in hypercalcemic sarcoidosis In the latter condition it may even accentuate the hypercalcemia (43) Despite this fact no special attention has been paid to the alkalosis

Both our patients who were studied during normal calcium intake were characterized by metabolic alkalosis hypokalemia and *low* urinary calcium excretion despite hypercalcemia and practically normal CCr In spite of the presumed suppression of parathyroid hormone secretion the TRCa % was even higher than in any case of primary hyperparathyroidism (Fig 5) This must be attributed to a specific effect of the electrolyte disturbance on the TRCa The fact tha induced metabolic *acidosis* (which is associated with a considerable potassium depletion) *reduces* TRCa and tends to *reduce* UFCa in normal man indicates the importance of the alkalosis (Transbøl Hahnemann and Thomsen unpublished) On the other hand the presence of a nearly comparable alkalosis in cases no 18 23 and 24 which had no hypokalemia had no apparent effect on TRCa % (Table I) If anything this might be taken as an indication that hypokalemia is of primary importance Unfortunately

it is very difficult to study separately the individ ual roles played by alkalosis and hypokalemia Both metabolic alkalosis and potassium depletion are common features of the milk alkali syndrome thus our observations implicate that an increased tubular reabsorption of calcium may contribute to the development and maintenance of hyper calcemia in the milk alkali syndrome It follows that TRCa % cannot be used for the differentia tion between primary hyperparathyroidism and the condition in question

Hypercalcemic States Associated with Depressed Levels of TRCa %

In agreement with the well established interplay between the parathyroid hormone secretion and the Ca^{++} a functional hypoparathyroidism lead ing to a depression of TRCa % must be expected in non parathyroid hypercalcemia (the milk alkali syndrome excepted) (57) It has been found in normal man that hypercalcemia caused by cal cium infusion reduces the TRCa (4 38) and in animals Sherwood et al (51) showed that in duced hypercalcemia inhibited the secretion of parathyroid hormone The combination of these observations affords the first experimental con firmation of the basic idea in the present work

Poststrumectomy hypoparathyroidism overdosed with vitamin D

All three patients presented moderate hypercal cemia reduced CCr hypercalcuria and consider ably decreased TRCa as also described by Canary et al (12) When increasing the concen tration of calcium in serum in a patient with un treated hypoparathyroidism hypercalcuria and reduction of the TRCa % occurs even before normocalcemia is obtained This takes place dur ing i v calcium infusion (4 16) as well as during vitamin D treatment (4 21 22 38) and thus it cannot be attributed to any specific effect of vitamin D on the tubular reabsorption of cal cium

Ordinary hypercalcemic sarcoidosis

The disturbance of calcium metabolism in sarcoid osis is attributed to intestinal hyperabsorption of calcium (1 28) As hypercalcuria with or without elevation of the serum total calcium con centration may occur *in spite of* normoabsorp tion it has been claimed that sarcoidosis may exert a specific effect on the TRCa % or on the bones (12 27 35) However liberation of cal cium from metastatic calcifications may also be responsible (32) Calcium balance and tracer studies revealed hyperabsorption of calcium in cases no 19 and 22 while in case no 23 where the patient was examined in the course of a spontaneous remission absorption was normal (32) All five patients had reduced TRCa % analogous with the observations of Canary et al (12) and also decreased tubular reabsorption of glucose as previously mentioned (58) The latter finding agrees with our assumption that the de crease in the TRCa is due to functional hypo parathyroidism (57) Hypercalcuria in presence of a normal concentration of total calcium in serum may be explained in the same way as Ca^{++} in sarcoidosis frequently is found raised with normal total calcium concentrations (24) The complex bound serum calcium fraction is also frequently raised in hypercalcemic sarcoidosis (24) but as this is also the case in some conditions with in creased TRCa the importance of the complex bound fraction for the tubular reabsorption of calcium is not clarified

Idiopathic intestinal hyperabsorption hypercalcemia

This newly recognized entity is characterized by cortisone resistant intestinal hyperabsorption of calcium hypercalcemia normophosphatemia normal phosphate excretion index excessive hy percalcuria depressed TRCa % and TmG/GFR —ratio associated with histologically normal para thyroid glands Probably it represents an extreme variant of that type of idiopathic hypercalcuria which is attributed to a primary intestinal hyperabsorption of calcium (33) The low TRCa is believed to be due to suppression of the parathyroid hormone secretion A similar patient (subject A W) has been followed since 1953 by Pyrah (48) and subsequently by Hodgkinson and Edwards (31)

Hypercalcemia associated with Hodgkin s paragranuloma

This disease resembles sarcoidosis with regard to clinical course as well as to immunology (37) In case no 28 the similarity also included the disturbance of calcium metabolism The points of resemblance are 1 intestinal hyperabsorption of

calcium 2 a very variable hypercalcemia accentuated by exposition to sunlight and easily and completely suppressed by cortisone 3 normophosphatemia 4 reduced TRCa % (95 5%) and normal phosphate excretion index during border line hypercalcemia and 5 considerably reduced TRCa % (88 5%) and raised phosphate excretion index during a phase with moderate hypercalcemia and lowered CCr On two occasions the TmG/GFR was surprisingly found to be slightly raised 2 19 and 2 44 (normal 1 87–2 07) As a small dose of prednisone (10 mg/24 h) has kept the serum calcium concentration normal for the last six months no indication for neck exploration has been present till now As it is we temporarily group the patient as a case of non parathyroid hypercalcemia but without neck exploration it cannot be decided whether he is in fact analogous to the patient with parathyroid hyperplasia and sarcoidosis (case no 18)

Myelomatosis

The hypercalcemia in myelomatosis must be regarded as of non parathyroid origin as it is due to a calcium releasing effect on bone by malignant plasma cells (2) In agreement with this a reduced TRCa % was demonstrated by Myers (44) and in the present study

The Phosphate Excretion Index in Hypercalcemia

The phosphate excretion index was without differential diagnostic value (Fig 6) This agrees well with the experiences of other investigators with the various parameters of phosphate excretion (44 45 56) If the tubular reabsorption of phosphate—and with that the P E I—was mainly a function of the parathyroid hormone secretion *reduced* P E I could be expected in patients with non parathyroid hypercalcemia However reduction of P E I was never demonstrated The causes for the inapplicability of P E I with regard to differential diagnosis are 1 that *irrespective* of the cause of the hypercalcemia P E I is raised at CCr levels below 55–65 ml/min (Table I and Fig 6) —just as in renal insufficiency in general (19) 2 that the tubular reabsorption of phosphate is directly affected by the serum calcium concentration when the secretion of parathyroid hormone is reduced (16) and 3 that thyrocalcitonin seems to reduce the tubular reabsorption of phosphate (49)

Classification of Hypercalcemic Conditions According to the Functional State of the Parathyroid Glands

Only two parameters are necessary to classify the disorders of calcium metabolism in relation to the level of parathyroid activity One is the concentration of ionized calcium in serum and the other a measure of the secretion of parathyroid hormone When a disorder has been classified as hypercalcemic it would be preferable to measure the parathyroid activity by the concentration of parathyroid hormone in plasma or by the secretion rate of parathyroid hormone When direct measurements of parathyroid hormone are unattainable an indirect measure may be relied on The present study indicates that the TRCa % seems useful for this purpose Other useable measures include the cortisone test and the maximal tubular reabsorptive capacity for glucose TmG/GFR (25 58) while on the other hand the phosphate excretion index seems useless Concerning the TRCa it should be mentioned that none of our patients with primary hyperparathyroidism had such pronounced hypercalcuria (600–700 mg/24 h) as Hodgkinson found in a few patients in his material (30) Therefore our observations have to be reexamined in larger groups of patients before the final differential diagnostic value of the TRCa % can be said to be established

ACKNOWLEDGEMENTS

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REFERENCES

1 Anderson J Dent C E Harper C & Philpot G R Lancet 2 7 0 1954
2 Bentzel C J Carbone P P & Rosenberg, L J clin Invest 43 213, 1964
3 Bernstein D Kleeman C R Cutler R E Dowling, J T & Maxwell M H Proc Soc exp Biol Med 110 671 1962
4 Bernstein D Kleeman C R & Maxwell M H Proc Soc exp Biol Med 121 353 1963
5 Berson S A & Yalow R S Science 154 907 1966
6 Bett I M & Frazer G P Biochem J 68 13 1958
7 Better O Gonick H C Chapman L & Kleeman C R Clin Res 13 301 1965
8 Better O S Gonick H C Chapman L C Varrady P D & Kleeman C R Proc Soc exp Biol Med 121 592 1966

9 Briscoe A M & Ragan C Metabolism 15 100 1966
10 Burnett C H Commons R R Albright F & Howard J E New Engl J Med 40 787 1949
11 Canary J J & Kyle L H J clin Invest 38 994 1959 (Abstract)
1? Canary J J Mintz, D Prezio J & Meloni C Clin Res 1? 263 1964 (Abstract)
13 Carruthers B M Copp D H & McIntosh H W J Lab clin Med 63 959 1964
14 Chen P S & Neumann W F Amer J Physiol 180 6 3 1955
15 Edwards N A & Hodgkinson A Clin Sci 9 327 1965
16 Eisenberg, E J clin Invest 44 942, 1965
17 Flocks, R H J Amer med Ass 113 1466 1939
18 Frick A Rumrich C Ullrich K J & Lassiter W E Pflug Arch ges Physiol 286 109 1965
19 Goldman R & Bassett, S H J clin Invest 33 16 3 1954
20 Gomori, G J Lab clin. Med 27 955 1941
21 Gordan G S Loken H F Blum A & Teal J S Metabolism 11 94 196?
2? Gordan G S Eisenberg E Loken H F Gardner B & Hayashida, T Recent progress in hormone research 18 297 1962
23 Hahnemann S Lancet 2 855 1965
24 Hahnemann S Transbøl I & Hornum I In Proceedings of the IVth International Sarcoidosis Conference held in Paris 1966 In print
25 Halver B Acta med scand 181 209 1967
26 Hawk P B Oser B L & Summerson W H Practical physiological chemistry 12th ed p 50? McGraw Hill New York 1947
27 Hendrix, J Z. Ann intern. Med 64 797 1966
28 Henneman P H Dempsey E F Carroll E L & Albright F J clin Invest. 35 12?9 1956
29 Hodgkinson A & Pyrah L N Brit J Surg 46 10 1958
30 Hodgkinson A Clin Sci 25 ?31 1963
31 Hodgkinson A & Edward N A Brit J Urol 35 445 1963
3? Hornum I Transbøl I & Hahnemann S In Proceedings of the IVth International Sarcoidosis Conference held in Paris 1966 In print
33 Hornum, I Transbøl I Hahnemann, S & Halver B In Proceedings of the Vth European Symposium on Calcified Tissues 1967 In print
34 Howard P J Wilde W S & Malvin R L Amer J Physiol 197 337 1959
35 Jackson W P U & Dancaster C P J clin Endocr 19 658 1959
36 Jahan I & Pitts R F Amer J Physiol 155 4 1948
37 James D G Lancet 633 1966
38 Kleeman C R Bernstein D Rockney R Dowling J T & Maxwell M H Yale J Biol Med 34 1 1961
39 Kleeman C R Bohannan J Bernstein D Ling, S & Maxwell M H Proc Soc exp Biol Med 115 9 1964
40 Laake H Acta med scand 16? 71 1959
41 Lafferty F W & Pearson O H J clin Endocr 23 891 1963
4? Lassiter W E Gottschalk C W & Mylle M Amer J Physiol 204 771 1963
43 Mather G Brit med J 1 48 1957
44 Myers W P L Advanc intern Med 11 163 196
45 Nordin B E C & Fraser R Lancet 1 947 1960
46 Poulos P P J Lab clin Med 49 253 1957
47 Punsar S & Somer T Acta med scand 173 435 1963
48 Pyrah L N Proc Roy Soc Med 51 183 1958
49 Robinson C J Martin T J & McIntyre I Lancet 2 83 1966
50 Rose G A Clin chim acta 2 ? 7 1957
51 Sherwood L M Potts J T Care A D, Mayer G P & Aurbach G D Nature ?09 ?2 1966
52 Siggaard Andersen O Engel K Jørgensen K & Astrup P Scand J clin Lab Invest 1? 17? 1960
53 Talmage R V & Kraintz, L W Proc Soc exp Biol Med (N Y) 87 ?63 1954
54 Talmage R V Kraintz, F W & Buchanan, G D Proc Soc exp Biol Med (N Y) 88 600 1955
55 Tashjian, A H, Levine L & Munson P L J exp Med 119 467 1964
56 Thomas, W C Connor T B & Morgan H G New Engl J Med 260 591 1959
57 Transbøl I Hahnemann, S & Hornum, I In Proceedings of the IVth International Sarcoidosis Conference held in Paris 1966 In print
58 Transbøl I & Halver B J clin Endocr 27 1193 1967
59 Walser M & Robinson, B H B In Transfer of calcium and strontium across biological membranes (ed R H Wasserman) p 305 Academic Press New York and London 1963
60 Widrow S H & Levinsky N G J clin Invest 41 2151 196?
61 Wilkinson R H J clin Path 10 1?6 1957

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RENAL FUNCTION DURING CARDIAC PACEMAKING

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Abstract A hemodynamic and renal study was carried out on eight pacemaker treated patients with complete heart block At the time of study the patients were normotensive and well compensated Cardiac output brachial arterial pressure inulin and PAH-clearances as well as the excretion of electrolytes were determined on two or three selected heart rate levels between 45 118 for each patient during four to six 20 minute periods A change of the ventricular rate from low to high level gave a significant decrease of stroke volume and pulse pressure but cardiac output GFR and RPF were unchanged The most interesting result was the finding of a significantly increased sodium excretion and sodium potassium quotient on high heart rates Possible explanations of the mechanisms responsible for this action are discussed

Modern pacemaker treatment of patients with Adams Stokes syndrome due to complete heart block has been a therapeutic success (8 13 15) This method has also offered new possibilities to study the response of the cardiovascular system in man to changes in the pacemaker induced ventricular heart rate both at rest and during exercise

Such studies are of great interest not only in the individual patient in whom the ventricular heart rate giving the most favourable hemodynamic conditions should be aimed at but also in the physiological evaluation of the response in different sites of the peripheral vascular bed to changes in heart rate and cardiac output

Several studies have dealt with central hemodynamic changes during cardiac pacemaking (4 5 6 13 14 18 19 23) but comparatively few with renal splanchnic or cerebral blood flow (9 11 12 24 25)

This study was initiated in order to evaluate the effects in resting patients of changes in heart rate blood pressure and cardiac output on renal hemodynamics and the renal handling of water and electrolytes This cardio-renal interrelationship is also believed to be of interest for understanding the mechanisms involved in the early development of congestive heart failure (1 10 26 27)

MATERIAL

Eight patients with pacemaker-treated complete heart block were studied The patients were all informed about the purpose of the investigation and willing to participate Their clinical data are given in Table I None of the patients had any history of renal or hypertensive disease Patient 3 had a mild diabetes mellitus easily controlled by diet and without signs of glucosuria at the time of the study Except for arteriosclerosis of variable degree no other previous or present disease of importance for this study was found among the aged patients Three patients were on digitalis treatment as signs of congestive heart failure due to their heart disease had been noted, but at the time of the study all patients were well compensated and able to lie down flat during the three hours of the investigation without any cardiac discomfort. No other drugs were administered and no premedication was given before the study

The pacemaker implantations had been performed from 5 to 0 days prior to the studies, using an endocardial electrode in the right ventricle and an external pacemaker (Elema Schonander 138) for easy regulation of the heart rate

METHODS

Indwelling vascular catheters were used for blood sampling, pressure recordings and infusions Blood pressures were recorded by an Elema strain gauge manometer Mean pressures were obtained by electrical integration. Heart rates were calculated from the ECG

Glomerular filtration rate (GFR) and renal plasma flow (RPF) were estimated as the clearances of inulin and para amino-hippuric acid (PAH) using continuous infusion techniques and arterial blood sampling at the beginning and end of each period. Cardiac output was determined by a dye-dilution method using bromsulfalein (BSP) as indicator An indwelling bladder catheter was

Table I Clinical data for the material at the time of study

Pat no	Sex	Age	Known history of complete heart block (months)	Heart volume (ml/m² body surface)	Heart treatment	Serum creatinine (mg)
1	♀	73	14	520	Digitoxin 0 2 mg daily	1 1
2	♂	63	2	430	None	1 1
3	♂	70	2	600	Digitoxin 0 15 mg daily	0 9
4	♂	65	3	440	None	1 0
5	♂	58	5	540	None	0 7
6	♀	67	1	—	None	1 0
7	♂	73	4	570	Lanatoside C 0 5 mg daily	0 9
8	♂	56	6	480	None	0 8

used for urine collection and the bladder was rinsed with 100 ml distilled water and air In two cases catheterisations were performed of a renal vein for the determination of the renal extraction of PAH In these cases the PAH-concentration in blood was kept at a level of 5–6 mg in all other cases around 3 mg The details of the methods and techniques used have been earlier reported from this laboratory (7)

Procedure

All studies were performed in the morning with the patients in the recumbent position They were all in the fasting state and well hydrated After the priming doses of inulin and PAH a continuous infusion of the clearance substances was started and 45 minutes were allowed for reaching equilibrium During this time all patients were kept on a pacemaker rate of about seventy beats per minute In all cases two clearance periods of 20 minutes were run on each selected heart rate level In cases 1–5 the investigation started at a low pacemaker rate with a stepwise increase of the rate at the beginning of periods 3 and 5 so that in all six periods were run in succession In cases 6–8 two equally long periods on a high rate were followed by another two on a low rate making four periods in all Each patient was equipped with his own pacemaker battery allowing five fixed levels of heart rate varying however from one pacemaker to another We accepted this variability of heart rate rather than disturb the patients composure by changing pacemaker just before the study The heart rate levels were chosen so that the patients felt comfortable and no signs of interference appeared For these reasons the investigated frequency intervals are not of the same size in all patients

The renal extraction of PAH was determined twice a period in cases 6 and 7

Cardiac output was determined at the beginning of the second period at each selected heart rate level In case 8 however cardiac output was not determined due to a technical failure Brachial arterial blood pressures were registered 2–3 times in each period and immediately before and after the determinations of cardiac output Continuous ECG registration was used

Calculations

Renal clearances renal blood flow cardiac output as well as the renal fraction of cardiac output were calculated according to standard formulae (22) Vascular resistances are given as arbitrary units The mean values of all observations made during the two clearance periods on each heart rate level were used in the calculations except for the calculation of the systemic vascular resistance in which the pressures registered immediately before and after the determination of cardiac output were used In cases 6 and 7 the figures determined for the renal extraction of PAH were used in the calculations of the renal blood flow and renal fraction of cardiac output in the other cases the PAH-extraction was assumed to be 90% The renal venous pressure was always set to zero as none of the patients had clinical signs of elevated systemic venous pressure

Differences between mean values for the first and the last two periods for each parameter were analysed by T test

We considered this method justified although the heart rate levels for the patients were not identical as it can not be assumed that identical heart rates give corresponding hemodynamic or renal function in these patients with different heart size and myocardial condition The changes were calculated in per cent for each parameter except for systemic vascular resistance renal resistance renal fraction of cardiac output and Na/K quotient

RESULTS

Fig 1 shows the findings in one representative case (no 3) and data for all the material are given in Tables II and III with statistics In cases 6–8 the sequence of the clearance periods is reversed in the tables in order to facilitate the comparison of data from low to high heart rate levels with the other cases

All patients were within fairly normal ranges of arterial blood pressure with regard to their

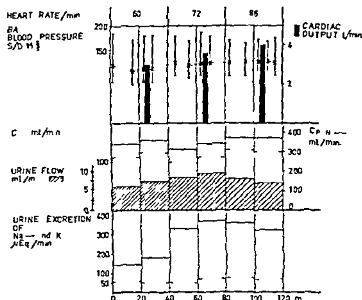


Fig 1 The basal hemodynamic and renal findings in case 3 Two clearance periods were run on each selected heart rate of 60 7_ and 86 beats per minute

ages The cardiac output was slightly reduced when studied at the lowest heart rate levels GFR and RPF were subnormal in all patients except nos 3 and 5 The mean value of the lowest heart rate level for the whole group was 56 and of the highest 96 beats p r minute

Table II *Hemodynamic data for the material*

In cases 6–8 the s-qu-nce of the clearanc- periods is reversed

Pat. no	Clearance period	Heart rate (/min)	B P mm Hg Brach art. S/D	Mean	Cardiac output (l min)	Stroke volume (ml)	Syst vasc res st (arb units)	Hematocrit (%)
1	1–2	48	186/74	110	_9	61	37 2	41
	3–4	75	173 87	118	3 4	45	35 9	42
	5–6	89	170/96	12_	3 1	35	39 7	42
2	1–_	65	13 /81	104	3 9	60	_6 7	38
	3–4	95	140/91	110	4.2	47	?6 4	38
	5–6	118	138/95	109	4 1	34	25 9	38
3	1–	60	178 81	109	3 0	51	36 7	40
	3–4	72	177/96	1_0	3 6	50	33 9	40
	5–6	86	168/95	123	4 0	46	30 8	39
4	1–_	45	152/73	93	4 1	91	22.6	38
	3–4	71	145/78	101	4 5	63	22.7	38
	5–6	86	15_ 85	104	4 6	65	_2.8	39
5	1 ?	48	167/81	105	4 5	94	23 6	44
	3–4	74	149 90	103	4 9	66	21 0	44
	5–6	87	147/96	105	4 9	56	21 4	44
6	3–4	68	161/85	105	3 7	54	28 1	42
	1–_	99	138/84	100	3 7	37	27 6	43
7	3–4	68	12_ 67	86	4 1	63	21 0	44
	1–_	100	1?3/75	91	3 8	4	_4 5	44
8	3–4	48	156/79	115				
	1–_	105	141/91	110				
Mean change from lowest to highest heart rate			Pulse pressure −25 9*	+4 5	+8 4*	−34 3*	0.5	
	S E.		3 7	2.5	4 8	4 4	1 2	
	P		<0 001			<0 001		

Table III *Renal data for the material*

In cases 6–8 the sequence of the clearance periods is reversed Case 5 was excluded *from the statistical* calculations (for reasons see text)

Pat no	Clearance period	Heart rate (/min)	Urine flow (ml/min)	Inulin clearance (ml/min)	PAH clearance (ml/min)	Renal extraction of PAH ( )	Na (μEq/min)	K (μEq/min)	Na/K	Total renal resistance (arb units)	Renal fraction of cardiac output ( )
1	1–2	48	4 0	59	225	—	105	34	3 1	259	14 6
	3–4	75	3 7	70	277	—	176	41	4 3	222	15 6
	5–6	89	2 1	55	212	—	178	38	4 7	300	13 1
2	1–2	65	9 2	95	416	—	179	104	1 7	140	19 1
	3–4	95	7 6	88	398	—	192	91	2 1	154	17 0
	5–6	118	5 5	78	332	—	209	81	2 6	183	14 5
3	1–2	60	6 5	113	350	—	117	56	2 1	168	21 6
	3–4	7	8 6	103	327	—	355	58	5 8	198	16 8
	5 6	86	7 3	122	390	—	360	71	5 1	173	17 8
4	1–2	45	3 2	88	403	—	74	79	0 9	129	17 6
	3–4	71	4 2	91	436	—	118	70	1 7	129	17 4
	5 6	86	3 4	89	429	—	122	65	1 9	133	17 0
5	1–2	48	2 3	194	7 9	—	113	56	2 0	73	32 2
	3–4	74	4 1	127	45[illegible]	—	91	52	1 8	115	18 3
	5–6	8	0 7	113	376	—	76	40	1 9	141	15 2
6	3–4	68	1 0	77	289	91	133	76	1 8	194	14 6
	1–2	99	2 9	84	305	92	140	75	1 9	172	15 7
7	3–4	68	3 1	66	265	88	188	59	3 2	162	13 0
	1–2	100	9 8	73	287	89	272	60	4 5	158	15 1
8	3–4	48	1 9	96	465	—	140	81	1 7		
	1–2	105	5 7	114	627	—	261	116	2 3		
Mean change from lowest to highest heart rate			+52 3	+3 5	+5 8		+72 3	+6 9	+1 21	+11 2	−1 2
	S E		41 3	4 7	6 3		27 4	8 5	0 35	10 5	1 08
	P						<0 05		<0 01		

Central hemodynamics

In all patients the ventricular heart rate was strictly conducted by the pacemaker activity at the rate levels selected and no interfering ventric ular premature beats were observed A change of the ventricular heart rate from low to high level was accompanied by a statistically significant narrowing of the pulse pressure averaging 26% The mean arterial blood pressure did not change however Cardiac output slightly increased in a few patients on increase of the heart rate from the lowest rate levels but this change was not statistically significant. The stroke volume con sequently decreased The systemic vascular re sistance remained unchanged

Renal effects

Large individual variations in the urine flow were observed but with no relation to the heart rate in the whole material

GFR and RPF showed only minor changes with an unchanged filtration fraction The total renal vascular resistance and the renal fraction of the cardiac output were similarly unchang d

Sodium excretion increased significantly (72°) when heart rate changed from lowest to highest level As potassium excretion remained un changed the Na/K quotient significantly in creased In the two patients in whom the renal extraction of PAH was determined normal figures were found and no change was observed at dif ferent rate levels In one patient (no 5) there was an obvious error in the renal clearance data giving suspiciously high figures for RPF and GFR in p riods 1–4 This patient had a low and rapidly changing urine flow and the high figures for GFR and RPF are probably due to inadequate washout of the urinary tract Whether or not this case was included in the statistical calculations the conclusions were the same We did not in clude this case in our calculations of the renal parameters but report his mean data in order to keep to the sequence of the investigation

DISCUSSION

Central hemodynamics

The idioventricular bradycardia of complete heart blo k is frequently accompanied by a low cardiac output (4 25) Increasing the ventricular rate to 50–60 by ventricular pacing ususally improves the cardiac output significantly A further in crease however from 60–120 often does not augment the cardiac output at all or results in a small enlargement Usually the most favorable cardiac output is found between 70–90 beats per minute Sowton (23) found a correlation between such peaked curves" and evidence of myocardial disease in the patients When the myocardium is seriously damaged its ability to keep up a high cardiac output is reduced to a limited heart rate interval and outside this the cardiac output will fall

In this study no significant change in cardiac output was found between the lowest and the highest heart rate levels As the number of pa tients studied was rather small we did not attempt to correlate the cardiac output curves to the present signs of myocardial disease Our findings seem however to be in good accordance with earlier reports showing above all that if there is a maximal cardiac output, it usually will be found between the heart rates of 70–90 beats per minute

As cardiac output did not significantly change when the heart rate was increased there was a pronounced decrease in the stroke volume and pulse pressures The mean arterial blood pressure and the calculated systemic vascular resistance showed only insignificant changes within the rate range studied

Renal hemodynamics

The glomerular filtration rate and the renal plasma flow may increase with pacemaker treat ment as compared to the situation with idio-ventricular bradycardia. This has been observed by Schuller et al (20)

In this study the GFR and RPF remained un changed when the ventricular heart rate was in creased This is in fact not surprising as the filtration pressure was found to be essentially unchanged The variation in ventricular stroke volume and pulse pressure obviously had no in fluence on the GFR and RPF In the two cases in which the renal extraction of PAH was deter mined no changes were observed indicating that the PAH-clearance is a valid measure of the renal plasma flow in this study Individual variations in cardiac output at different heart rate levels were not accompanied by similar changes in renal blood flow

Changes in ventricular stroke volume and pulse pressure obviously did not influence the renal hemodynamics suggesting an effective autoregulation mechanism Our results are in agreement with those of Humphries et al (12) who noted unchanged GFR RPF and cardiac output in pacemaker treated patients with complete heart block when changing their heart rate from 40–70 beats per minute

The renal handling of water and electrolytes

The urine flow varied considerably in different patients and was not influenced by the heart rate *It is difficult in studies like this to achieve a* constant hydration state as a rapid intravenous infusion might be hasardous to the circulatory state

The most striking change found in this study was an increase of the renal excretion of sodium as well as the sodium/potassium quotient when the heart rate was increased The increase of sodium excretion however did not correspond to the increase of heart rate as seen in cases 1 2 and 4 Serum electrolytes were all unchanged during the studies (data not published) and as the GFR was also unchanged the increased sodium output could not be demonstrated to be due to an increase in the filtered load of sodium

D kker et al (9) observed a similar chang in the sodium excretion in one patient with pacemaker when the heart rate was increased from 50–100 beats per minute No simultaneous clearance studies were performed Friedberg et al (11) studied the ability to excrete graded loads of sodium in four patients with total heart block before and after pacemaker treatment They found a significant increase of the maximal excretion of sodium when the heart rate was returned to normal but no change in the glomerular filtration rate

The increase in the Na/K quotient in this study raises the question whether the observed change has been mediated by an aldosterone mechanism The decrease in ventricular stroke volume and arterial pulse pressure might possibly activate intrarenal receptors and the angiotensin aldosterone system or even exert a direct influence on the adrenal cortex

Although changes in the Na/K quotient throw suspicion on an altered aldosterone activity there are certain arguments against such a mechanism in this study

It is well known that there is always a delay of at least 30–60 minutes after the administration of aldosterone until the effect on electrolytes is observed even if the hormone is injected directly into one renal artery (3) Sharp et al observed (21) that exposure of a toad bladder to aldosterone for only five minutes followed by washing elicited a stimulation of the sodium transport reaching its maximum two hours after the washing procedure It does not seem probable that a change in the aldosterone activity rate could account for the change in sodium excretion within 20 minutes as was found in this study whether the heart rate was changed from low to high or vice versa A possibility remains that the changes in sodium output might be due to angiotensin which is presumed to exert a local action on the tubular reabsorption mechanism (16 17)

Barger et al (2) showed that changes in the intrarenal distribution of blood can affect the excretion of sodium An increased outer medullary flow was accompanied by a decrease in sodium excretion Such changes in the renal blood distribution may occur without measurable changes in the GFR or RPF

Whether changes in pulse pressure or stroke volume might affect the renin angiotensin system or the intrarenal blood distribution via other intrarenal receptor systems is an interesting question that still remains to be solved

REFERENCES

1 Barger A C Muldowney F P & Liebowitz M R Role of the kidney in the pathogenesis of congestive heart failure Circulation 20 273 1959
2 Barger A C & Held J A Study of renal circulation in the unaesthetized dog with inert gases 1 External counting Abstracts International Congress of Nephrology p 95 1966
3 Barger A C Berlin R D & Tulenko J F Infusion of aldosterone 9 α fluorhydrocortisone and antidiuretic hormone into the renal artery of normal and adrenalectomized unaesthetized dogs effects on electrolyte and water excretion Endocrinology 6 804 1958
4 Benchimol A Cardiac functions during electrical stimulation of the heart Amer J Cardiol 17 7 1966
5 Benchimol A Ellis J G & Dimond E G Hemodynamic consequences of atrial and ventricular pacing in patients with normal and abnormal h arts Amer J Med 39 911 1965

6 Bevegård S Observations on the effe t of varying ventricular rate on the circulation at rest and during exercise in two patients with an artificial pacemaker Acta med scand 172 615 1962
7 Bojs G An experimental study on seroton n on man with special reference to renal function Acta med scand Suppl 55 1961
8 Carleton R A Sessions R. W & Graettinger J S Card ac pacemakers clinical and physiologi al stud ies Med Clin N Amer 50 3 5 1966
9 Dekker E., Meerschwam, I S Buller J & Pel er H E The influence of electronically induced heart rate on the excretion of minerals and water in a pa tient with total heart block. Israel med J ⌄ 365 1963
10 Forland M & Pullman T N Renal aspects of cardiac disease Med Clin c N Amer 50 255 1966
11 Friedberg, C K. Donoso E., Stein W G Kahn M & Litwak R The role of bradycard a in the retent on of sodium and water in complete heart block with and without heart failure in human beings Amer Heart J 69 293 1965
1? Humphries, J O., Hinman, E J Ueda K. & Walker W G The influence of heart rate on cardio-renal function Clin Res 10 406 196?
13 Johansson B W Complete heart block A clinical hemodynamic and pharmacological study in patients with and without an artificial pacemaker Acta med scand Suppl 451 1966
14 Judge R. D Wilson W S & S egel J H Hemodynamic studies in patients with implanted cardiac pacemakers New Engl J Med 270 1391 1964
15 Lagergren H Johansson L Schuller H Kugel berg J., Bojs G Alestig, K., Borst, H G Schaud g, A G ebel O Harms H Rodewald G & Schep- pokat, K. D 305 cases of permanent intravenous pacemaker treatment for Adams Stokes syndrome Surgery 59 49? 1966
16 Leyssa P P Intrarenal function of angiotensin Fed Proc 6 55 1967
17 McGiff J C Natriuretic effect of angiotensin in dogs revealed after administration of reserpine and guanethidine Circulat Res 20 664 1967
18 McGregor M & Klassen G A Observations on the effect of heart rate on card ac output in patients with complete heart block at rest and during exer cise Circulat Res Suppl 2 215 1964
19 Samet P Bernstein W H Levine S & Lopez, A Hemodynamic effects of tachycardias produced by atrial and ventricular pacing Amer J Med 39 905 1965
?0 Schuller H Tryding, N & Westling H Die Nieren funktion bei totalem AV Block vor und nach Pace makerbehandlung Thoraxchirurgie 12 189 1964
?1 Sharp W G & Leaf A Studies on the mode of action of aldosterone Recent Progr Hormone Res ? 431 1966
? Smith, H W The kidney Principles of renal phys ol ogy Oxford University Press New York 1956
?3 Sowton, E Hemodynamic studies in patients with art ficial pacemakers Brit Heart J 6 737 1964
?4 Sulg, I A EEG och cerebralt blodflode fore och efter inplantering av artificiell hjartpacemaker p 81 Medicinsk Riksstamma Stockholm 1966
25 Taylor A B Experience with cardiac pacemaking Brit med J 2 543 1966
26 Urquhart J & Davis J O Role of the kidney and the adrenal cortex in congestive heart failure I Mod Conc cardiov Dis 3 781 1963
27 — Role of the kidney and the adrenal cortex in congestive heart failure II Mod Conc cardiov Dis 32 787 1963

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INTESTINAL ABSORPTION AND AUTOIMMUNITY IN ENDOCRINE DISORDERS

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Abstract Thirty two patients with endocrine disorders were studied from gastroenterological and immunological points of view Gastritis was found in 18 patients It was atrophic in ten patients Two of the latter had pernicious anemia with idiopathic hypoparathyroidism Jejunal biopsy revealed a normal mucosa in 20 patients an increase of "inflammatory cells in four and villous alterations in six Three of the latter had spontaneous hypothyroidism and two signs of autoimmune thyroiditis A general malabsorption syndrome was present in eight patients one had only steatorrhea and one possibly selective malabsorption of vitamin B Of 19 patients with thyroid disease alone or in combination with other endocrine disorders seven showed signs of malabsorption six of whom also had villous alterations Villous alterations were associated with immunological disturbances except for one patient who had postoperative hypothyroidism They did not respond to endocrine treatment nor to treatment with gluten free diet except for the patient without immunological changes Distinct edema of muscularis mucosae was visible in 13 out of 16 patients with hypothyroidism Malabsorption and villous changes in thyroid disease may be due to 1 myxedematous changes 2 altered motility and 3 autoimmune phenomena Of 12 patients with hypoparathyroidism alone or in combination with other endocrine disorders four had signs of malabsorption but only one with concomitant hypothyroidism and high thyroid antibody titers had villous alterations Correction of calcium metabolism in three patients resulted in improvement of steatorrhea in all but the one with concomitant thyroid disease This may point to a purely functional relationship between absorption and calcium metabolism

Histological and functional changes in the stomach and in the small intestine before and after treatment in hyperthyroidism have been described by the authors in previous communications (32 33) The present report deals with gastrointestinal changes in other endocrine disorders especially in hypothyroidism autoimmune thyroiditis and hypoparathyroidism Such a study seemed to be of considerable interest firstly since alterations in the endocrine functions may produce changes in the functional capacity of the gastrointestinal tract and secondly since some endocrine diseases seem to have a common genetic and/or autoimmune background also postulated for some gastrointestinal disorders It is the purpose of the present communication to show the occurrence of some intestinal changes especially malabsorption in hypothyroidism autoimmune thyroiditis and hypoparathyroidism and to discuss possible functional and autoimmune relationships

MATERIAL AND METHODS

A number of patients admitted to the 1st 2nd and 3rd Departments of Medicine University of Helsinki during three consecutive years were studied However many patients refused the gastroenterological examinations and in several subjects the study had to be interrupted for various reasons Altogether there remained 32 patients for the final analysis The patients fall into several groups of endocrine disorders or combinations of them as shown in Table I Of the patients 0 were females and 12 men The mean age was 42 years (16–73 years)

The following gastroenterological examinations were carried out gastric biopsy 25 maximal histalog test 4 biopsy of the small intestine 30 determination of the fecal fat excretion according to the method of van de Kamer 3 the vitamin A absorption test 23 the *d* xylose tolerance test 28 and the Schilling test 18

The gastric and intestinal biopsies were taken with the Sielaff and the multipurpose suction biopsy tubes In all but two patients biopsy samples were obtained from the proximal jejunum In 5 patients 58 specimens were obtained from the body of the stomach The biopsy specimens were fixed in 10% neutral formalin and stained with hematoxylin-eosine and PAS

General immune responses were looked for by the aid of conventional paper electrophoresis and immune electrophoresis of the serum proteins Circulating thyroid antibodies were tested for with the red tanned cell hemagglutinin and the complement fixation technique Routinely

Table II *Some data on the patients with endocrine disease associated with pernicious anemia and malabsorption*

					Gastroenterological findings		
Pat	Sex	Age	Endocrine disease	Co-existing diseases	Gastric mucosa	Intestinal mucosa	Signs of malabsorption
T G	♀	61	Spontaneous hypothyroidism	—	Normal	Partial villous atrophy (Fig 1)	Only Schilling I and II pathological
S V	♀	62	Spontaneous hypothyroidism	—	Lymphocytic infiltration normal glands	Subtotal villous atrophy	Diarrhea fecal fat 29 g/day vitamin A d xylose and Schilling tests pathological
P M	♀	16	Spontaneous hypothyroidism	—	Not examined	Subtotal villous atrophy	Diarrhea vitamin A and d xylose tests pathological fecal fat not examined
A P	♀	24	Postoperative hypothyroidism	—	Moderate atrophic gastritis	Subtotal villous atrophy (Fig. 2)	Diarrhea fecal fat 18 g/day vitamin A and d xylose tests pathological
G B	♂	45	Postoperative hypothyroidism	—	Not examined	Granulomatous ileo-colitis	Diarrhea fecal fat 9 g/day vitamin A and d xylose tolerance tests pathological
K K	♀	47	Postoperative hypothyroidism and hypoparathyroidism	—	Normal	Subtotal villous atrophy (Fig 5)	Diarrhea fecal fat 19 g/day vitamin A and d xylose tolerance tests pathological
O J	♀	41	Asymptomatic thyroiditis	—	Normal	Subtotal villous atrophy	Diarrhea fecal fat 12 g/day vitamin A and d xylose tolerance tests pathological
E E	♀	45	Idiopathic hypoparathyroidism	Pernicious anemia diabetes	Gastric atrophy	Normal (Fig 7)	Diarrhea fecal fat 33 g/day vitamin A and d xylose tolerance tests pathological
M N	♂	32	Idiopathic hypoparathyroidism	Pernicious anemia	Gastric atrophy	Normal	No
L G	♂	62	Postoperative hypoparathyroidism	Waldenström s macroglobulinemia	Moderate atrophic gastritis with metaplasia	Normal	Diarrhea fecal fat 18 g/day vitamin A d xylose and FIGLU tests pathological
H H	♀	51	Parathyroid adenoma with Addison s disease	—	Not examined	Normal	Diarrhea fecal fat 23 g/day no other signs of malabsorption

changes were seen only in 2 out of 16 patients with other endocrine diseases In two hypothyroid patients in which the biopsy was repeated after adequate substitution therapy the edema disappeared No PAS positive material was observed in the muscularis mucosae contrary to the findings of Douglass and Jacobson (9) in necropsy specimens from myxedematous patients

Biopsy of the small intestine revealed subtotal villous atrophy (Figs 2 and 5) in five patients and partial villous atrophy (Fig 1) in one subject The distribution of these patients within the different groups is of some interest one patient had asymptomatic autoimmune thyroiditis three had spontaneous hypothyroidism (one of them with high thyroid antibody titers and one with cytologically evident thyroiditis) one had postoperative hypoparathyroidism and hypothyroidism with high thyroid antibody titers (K K) and only one had pure postoperative hypothyroidism without any signs of autoimmune thyroiditis All six patients also had an increased number of plasma cells and lymphocytes in the intestinal mucosa (Figs 2 and 5) Three additional patients with hypothyroidism showed an increase of these cells without epithelial changes (Fig 6)

of the small
ne to treatment

docrine	Gluten free diet	Comments
o	No	Presumably selective vitamin B_{12} malabsorption still under investigation partial villous atrophy not responding to treatment
nent still progress	Symptomatic only	Malabsorption and thyroid antibodies found in 1963 hypothyroidism developed in 1967 poor response to gluten free diet elevated thyroid antibodies
reatment st ll progress	Treatment still in progress	Malabsorption with subtotal villous atrophy the follow up still in progress biopsy lymphocytic thyroiditis
ormalization of estinal mucosa ig. 4) and absorp- tests	No	Malabsorption with villous atrophy respond ng to endocrine treatment during normal diet
	Not examined	Died of intest nal obstruction autopsy granulomatous entero-colitis
	No	Malabsorption with villous atrophy not responding to any treatment elevated thyroid antibodies
	No	Malabsorption with villous atrophy not responding to gluten free diet elevated thyroid antibodies
ormalization f absorption s	No definite effect	Began in early childhood as hypoparathyroidism malabsorption pernicious anemia and diabetes developed later on distinct malabsorption without morphological alteration respond ng to treatment of hypoparathyroid sm
ned	Not examined	Described in detail by Ikkala et al (14)
isappearance steatorrhea	Not examined	Malabsorption with a normal intestinal mucosa associated with Waldenstrom s macroglobulinemia and postoperative hypoparathyroidism disappearance of steatorrhea during endocrine treatment with a normal diet
ot ollowed up	Not examined	Steatorrhea without other signs of malabsorption with normal intestinal mucosa and normal pancreatic function

and one patient was found on operation to have granulomatous ileo-colitis Altogether 10 out of 32 patients had some anatomical changes in the small intestine

Closer examination of the intestinal biopsy specimens revealed normal amounts of Paneth cells in all specimens and no PAS-positive material or lymphangiectasiae in the lamina propria Generally no qualitative alterations were evident in the various specimens with villous changes In addition no essential differences could be demonstrated on comparison with specimens taken from patients with so-called gluten-enteropathy not associated with endocrine diseases

Some data on the patients with malabsorption and with pernicious anemia are compiled in Table II Absorption studies disclosed malabsorption in ten patients six of them had also changes in the structure of the villi and one had granulomatous ileo-colitis In eight patients there was a general malabsorptive pattern with steatorrhea one patient had only steatorrhea (H H) and one (T G hypothyroidism) only a pathological absorption of vitamin B_1 in the Schilling I and II tests It



Fig 2 Patient A P with postoperative hypothyroidism before treatment Subtotal villous atrophy with proliferation of the crypts and increase of the cell content in the lamina propria H E ×75



Fig 3 Patient A P with postoperative hypothyroidism before treatment Edema and thickening of muscularis mucosae visible H E ×2 5



Fig 4 Patient A P with postoperative hypothyroidism after adequate substitution therapy The mucosa including muscularis mucosae appears almost normal H E ×100

seemed that signs of malabsorption were often associated with spontaneous hypothyroidism and idiopathic hypoparathyroidism occurring in four out of eight such patients Malabsorption was however also observed in four other patients but all these had also some thyroid or parathyroid disturbances

Clinical signs of chronic pancreatitis were not observed The secretin test was carried out only in five patients with steatorrhea the bicarbonate concentration was normal in three and slightly decreased in two subjects

Of the ten patients with steatorrhea six were *re studied after correction of the endocrine failure* The fecal fat excretion became normal in three of the six patients (one with postoperative hypothyroidism one with postoperative hypoparathyroidism and Waldenstrom s macroglobulinemia and one with idiopathic hypoparathyroidism) The remaining three patients who showed no response had some thyroid disorder (one had spontaneous hypothyroidism one postoperative hypoparathy

Table III *Immunological features of patients with endocrine diseases associated with malab orption and pernicious anemia*

Pat.	Endocrine disease	Gastrointestinal signs	Serum immuno globulins	Thyroid antibod es	Intrinsic factor antibodies	Not organ specific antibodies	Antibodies against whole human gastric mucosa
T G	Spontaneous hypothyroidism	Partial villous atrophy malabsorption of vitamin B_1 normal gastric mucosa	Increase	Absent	Present	Absent	Absent
S V	Spontaneous hypothyroidism	Subtotal villous atrophy malabsorption lymphocytic infiltration of the gastric mucosa	Increase[b]	Present TRC 1 2 5 mill cf 1 64	Not examined	Not examined	Not examined
P M	Spontaneous hypothyroidism with lymphoid cell thyroiditis	Subtotal villous atrophy malabsorption	Decrease	Absent	Not examined	Not examined	Not examined
A P	Postoperative hypothyroidism	Subtotal villous atrophy malabsorption atrophic gastritis	Increase	Absent	Present	Absent	Absent
G B	Postoperative hypothyroidism	Granulomatous entero col tis malabsorption	Increase	Not examined	Not examined	Not examined	Not examined
K K	Postoperative hypothyroidism and hypoparathyroidism	Subtotal villous atrophy malabsorption normal gastric mucosa	Increase	Present TRC 1 2 5 mill	Present (?)	Absent	Absent
O J	Asymptomatic thyroiditis	Subtotal villous atrophy malabsorption normal gastric mucosa	Increase	Present TRC 1 2 5 mill cf 1 4	Not examined	Present	Present
E E	Idiopathic hypoparathyroidism diabetes	Pernicious anemia with gastric atrophy malab sorption normal jejunal mucosa	Normal	Absent	Present	Absent	Ab ent
M N	Id opathic hypoparathyroidism	Pernicious anemia and gastric atrophy	Not examined	Not examined	Not examined	Absent	Absent

Fine needle biopsy lymphocytic thyroiditis
[b] Normalized after treatment with thyroid hormones

roidism and hypothyroidism with high antibody titers (K K) and one asymptomatic autoimmune thyroiditis) Additional trials with gluten free diet in these three patients did not improve the steatorrhea

The changes in the intestinal villi were re studied in four patients after periods of from four months to two years of substitution therapy for the endocrine disease In only one patient the villous changes disappeared (A P postoperative hypothyroidism) (Figs 2 3 4) In the remaining three patients there was also no change in response to a glutenfree diet regimen All three patients seemed to have normal amounts of Paneth cells All patients were thyroid cases (Table II)

The results from the immunological examinations are compiled in Table III The studies were carried out in the patients listed in Table II i e those with morphological changes in the small intestine and with pernicious anemia An increase of the immunoglobulins was found in 6 and a decrease in one out of 8 patients Thyroid antibodies were present in the blood of three patients (one had asymptomatic thyroiditis one spontaneous hypothyroidism and one postoperative hypoparathyroidism and hypothyroidism with high thyroid antibody titers (K K)) Intrinsic factor antibodies were found in the sera of 2 out of 4 patients In one of the patients (K K) the result was not conclusive Parietal cell antibodi s were detected only in the serum of a patient with asymptomatic thyroiditis (O J) Antibodies which were not organ specific (antinuclear factor positive Waaler Rose and latex fixation tests) were present only in the serum of this patient Also gastric body mucosa antibodies were only found in this patient

From the tables it appears that out of the 19



Fig 2 Patient A P with postoperative hypothyroidism *before treatment Subtotal villous atrophy with prolifera*tion of the crypts and increase of the cell content in the *lamina propria* H E ×75



Fig 3 Patient A P with *postoperative hypothyroidism* before treatment Edema and thickening of muscularis mucosae visible H E ×275



Fig 4 Patient A P with postoperative hypothyroidism after adequate substitution therapy The mucosa including muscularis mucosae appears almost normal H E ×100

seemed that signs of malabsorption were often associated with spontaneous hypothyroidism and idiopathic hypoparathyroidism occurring in four out of eight such patients Malabsorption was however also observed in four other patients but all these had also some thyroid or parathyroid disturbances

Clinical signs of chronic pancreatitis were not observed The secretin test was carried out only in five patients with steatorrhea the bicarbonate concentration was normal in three and slightly decreased *in two subjects*

Of the ten patients with steatorrhea six were re studied after correction of the endocrine failure The fecal fat excretion became normal in three of the six patients (one with postoperative *hypothyroidism one with postoperative* hypoparathyroidism and Waldenstrom s macroglobulinemia and one with idiopathic hypoparathyroidism) The remaining three patients who showed no response had some thyroid disorder (one had spontaneous hypothyroidism one postoperative hypoparathy

was seen The only patient who did not respond was the patient with concomitant postoperative hypothyroidism and high thyroid antibody titers (K K)

No signs of malabsorption were observed in other endocrine diseases (hypopituitarism Cushing s syndrome and Addison s disease)

DISCUSSION

The association of various thyroid diseases (autoimmune thyroiditis hypothyroidism and hyperthyroidism) Addison s disease hypoparathyroidism pernicious anemia atrophic gastritis and possibly diabetes mellitus is presently of outstanding interest In thyroid diseases gastritis (17 32 33) pernicious anemia (8 16 18 31 34 35) Addison s disease (4 5 15) and diabete mellitus (1 2 5 21 22) are more prevalent than in the normal population Antibodies against parietal cells of the stomach and intrinsic factor (8 10 17 18 31 37) and adrenal tissue (15 19) are more common findings than in the average population A common possibly genetically determined autoimmune background has been suggested (19) The same applies to idiopathic hypoparathyroidism in which there seems to be some association with pernicious anemia and Addison s disease (3 13 14 24) The present series of 32 patients with various endocrine diseases in various combinations may hence be of some interest

Gastric changes

The common occurrence of gastritis (70% of the cases) is also of some interest since the gastritis was atrophic in 40% (10 patients) Pernicious anemia was present in three of those ten patients and was associated either with spontaneous hypothyroidism or idiopathic hypoparathyroidism One of the cases in the last mentioned group (M N) has been previously reported (14) In these cases adequate correction of the calcium metabolism did not influence the absorption of vitamin B_1 It is hardly conceivable therefore that the alterations in calcium metabolism induced vitamin B_1 malabsorption A common background for these two conditions may therefore be postulated Furthermore the simultaneous occurrence of idiopathic hypoparathyroidism pernicious anemia intestinal malabsorption and diabetes mellitus in one case (E E) rather points in the same direction

Small intestine

Less is known about the relationships between endocrine disorders and the function of the small intestine Signs of malabsorption were observed in ten patients including one patient possibly with selective malabsorption of vitamin B_1 (T G) In six of these patients morphological changes in the intestinal villi were observed in one patient the changes were only slight In the remaining four all of whom suffered from parathyroid disease the structure of the villi was entirely normal

Of the 19 patients with thyroid disease seven showed signs of malabsorption It might be significant as already mentioned under the Results that in four of these patients (S V P M K K and O J) there were signs of autoimmune thyroiditis that one had spontaneous hypothyroidism most probably due to autoimmune thyroiditis although no circulating antibodies were detected Of the remaining 14 patients only two with postoperative hypothyroidism had signs of malabsorption one of them also had granulomatous enterocolitis In five of the seven patients with malabsorption the intestinal biopsy showed subtotal villous atrophy (S V P M A P K K and O J) four of whom had signs of autoimmune thyroiditis and one pure postoperative hypothyroidism Partial villous atrophy was seen in one case (T G) with spontaneous hypothyroidism and vitamin B_1 malabsorption and one (G B) had granulomatous enterocolitis

Unfortunately only a very limited number of patients could be adequately restudied after treatment However the only patient in whom the malabsorption and the villous changes showed a definite response was one with pure" postoperative hypothyroidism (A P) indicating perhaps that the changes may have been induced by intestinal myxedema" In the three other patients there was no response either to treatment with thyroid hormones or to gluten free diet Two of these had evidence of autoimmune thyroiditis (K K and O J) and one had spontaneous hypothyroidism (T G) with vitamin B_1 malabsorption

According to the literature the association of malabsorption and hypothyroidism is uncommon

Kelley and Stewart (23) described a patient with this combination and with intestinal hypomotility in which the signs of malabsorption did not re spond to treatment with thyroid hormones Salmi and Lahesmaa (27) drew attention to the common occurrence of gastrointestinal troubles in infantile hypothyroidism which they supposed to be due to myxedematous changes in the gastrointestinal tract The possibility of myxedematous changes being the cause of various gastrointestinal disturb ances is fairly acceptable in view of the myx edematous alterations observed in most patients with hypothyroidism in this series But on the other hand one is apt to regard the prevalence of autoimmune manifestations in hypothyroidism accompanied by malabsorption and villous changes as being of significance

Of the 11 patients with hypoparathyroidism three showed signs of malabsorption The in testinal mucosa was abnormal only in one of them and this case had also hypoparathyroidism with high thyroid antibody titers (K K) When the endocrine disorder was adequately corrected the malabsorption disappeared in two patients but not in case K K This suggests that the mal absorption in the two cases without villous changes might have been in some obscure way due to the alterations in calcium metabolism

The fairly common co existence of malabsorp tion and idiopathic hypoparathyroidism has been stressed by several authors (6 11 20 26 28 30 36 38) Jackson (20) believes that this combina tion is a definite syndrome although he was not able to offer a satisfactory explanation Clarkson et al (6) on the other hand are of the opinion that the steatorrhea is secondary to the endocrine disorder In the patient they reported on the steatorrhea disappeared after adequate control of the calcium metabolism with vitamin D or para thyroid hormone Similar experiences have been reported by others (24) This is well in keeping with the observations in the present series and also with that of Visakorpi and Gerber (36) in an infant with hypoparathyroidism malabsorp tion and pernicious anemia Morse et al (26) sug gested that the combination of malabsorption and hypoparathyroidism is only a variant of muco viscidosis These authors observed an increased sodium concentration in the sweat in one of five siblings with idiopathic hypoparathyroidism Sal vesen and Bøe (28 30) have expressed the view that the signs of hypoparathyroidism in steator rhea may be due to the lack of some exogenous factor necessary for normal parathyroid function In one of their patients candida albicans was cultured in the feces and according to these authors this infection may have explained the presence of steatorrhea Moniliasis is a common complication in this syndrome (29)

Malabsorption on the other hand is not com mon in postoperative hypoparathyroidism In the two patients in the present series who had post operative hypoparathyroidism and malabsorption other complicating conditions were also present One had hypothyroidism with high thyroid anti body titers (K K) and the other had Walden strom s macroglobulinemia (L G) known to be associated with alterations in gastrointestinal functions This would point to the view that the steatorrhea in idiopathic hypoparathyroidism is in some way associated with the endocrine dis order and may become manifest through the derangement of the calcium metabolism This would suggest the existence of a real syndrome as suggested by Jackson (20)

Steatorrhea also occurred in a patient with primary hyperparathyroidism due to parathyroid adenoma and Addison s disease The three other patients with Addison s disease in the present series had however no signs of malabsorption It is not possible to offer an explanation of the malabsorption in this particular case

General immunological phenomena were com mon in this series of patients with malabsorption The immunoglobulins were increased in five pa tients significantly elevated thyroid antibodies and cytologically proved autoimmune thyroiditis were found in four patients intrinsic factor antibodies were observed in two out of four patients The most striking pattern was seen in one patient with asymptomatic autoimmune thyroiditis (O J) who had elevated thyroid antibodies positive latex fixation gastric and nuclear antibodies villous atrophy of the small intestine some arthritic features and recurrent deep thromboses This sug gests that some autoimmune mechanism may be operative in the production of malabsorption and structural changes in the small intestine in associa tion with some endocrine diseases The increase of lymphocytes and plasma cells in the intestinal mucosa may also be of importance and may have some relation to the findings of Crabbe et al

(7) according to which most intestinal plasma cells produce various immunoglobulins

On the evidence of the findings in the present series of patients it may be tentatively concluded that both changes in the endocrine functions and general autoimmune processes may be operative in the production of malabsorption In hypothyroidism malabsorption and steatorrhea may be due to (*a*) myxedematous changes and associated metabolic alterations in the intestinal mucosa (reversible) (*b*) altered intestinal motility (reversible) and (*c*) general autoimmune phenomena (irreversible) In idiopathic hypoparathyroidism the steatorrhea which is not associated with morphological changes in the intestinal mucosa is corrected by adequate treatment of the changes in calcium metabolism This may point to a purely functional relationship between the intestinal absorption and the calcium metabolism or the parathyroid hormone It is of interest to note that no improvement of the malabsorption or of the villous changes occurred in response to gluten free diet in those patients who did not respond to endocrine correction in this respect This suggests that gluten intolerance is usually not involved in this type of malabsorption associated with endocrine disorders

ACKNOWLEDGEMENT

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REFERENCES

1 Abt A F Hyperthyroidism and diabetes Metabolism 11 02 196

Andreani D Menzinger G Pinhera A Fallucca, F & Alberti G Diabetes in the families of patients with thyroid disorders (abstr) Acta endocr (Kbh) Suppl 119 71 1967

3 Blizzard R M Chee D & Davis W The incidence of parathyroid and other antibodies in the sera of patients with idiopathic hypoparathyroidism Clin exp Immunol 1 119 1966

4 Blizzard R M & Kyle M Studies on the adrenal antigens and antibodies in Addison's disease J clin Invest 42 1653 1963

5 Carpenter C C J Solomon N Silverberg, S G Bledsoe T Northcutt R C Klinenberg J R Bennett I L & Harvey A McG Schmidt's syndrome (thyroid and adrenal insufficiency) A review of the literature and a report of fifteen new cases including ten instances of co-existing diabetes mellitus Medicine (Baltimore) 43 153 1964

6 Clarkson B Kowlessar O D Horwith M & Sleisenger M H Clinical and metabolic study of a patient with malabsorption and hypoparathyroidism Metabolism 9 1093 1960

7 Crabbe P A & Heremans J F The distribution of immunoglobulin-containing cells along the human gastrointestinal tract Gastroenterology 51 305 1966

8 Doniach D Roitt I M & Taylor K B Autoimmunity in pernicious anaemia and thyroiditis a family study Ann N Y Acad Sci 124 605 1965

9 Douglass P C & Jacobson S D Pathologic changes in adult myxedema Survey of 10 necropsies J clin Endocr 17 1354 1957

10 Evans H A W Woodrow J C McDougall C D M Chew A R & Evans R W Antibodies in the families of thyrotoxic patients Lancet 1 636 1967

11 Fourman P & Haapanen E Parathyroid function in steatorrhoea with osteomalacia Schweiz med Wschr 94 886 1964

12 Haas H G Knochenstoffwechsel und Parathyreoidea Erkrankungen Thieme Verlag Stuttgart 1966

13 Hung W Migeon C J & Parrott R H A possible basis for Addison's disease in three siblings one with idiopathic hypoparathyroidism pernicious anaemia and superficial moniliasis New England J Med 269 658 1963

14 Ikkala E Siurala M & Viranko M Hypoparathyroidism and pernicious anaemia Acta med scand 176 73 1964

15 Irvine W J Autoimmune mechanisms in thyroid disease In The thyroid and its diseases (ed A Stuart Mason) p 129 Pitman London 1963

16 Irvine W J Davies S H, Delamore I W & Williams A W Immunological relationship between pernicious anaemia and thyroid disease Brit med J 2 454 196

17 Irvine W J Davies S H & Sumerling M D The immunopathy of thyroid disease In Current topics in thyroid research (eds C Cassano and M Andreoli) p 773 Academic Press New York 1965

18 Irvine W J Davies S H Teitelbaum S Delamore I W & Williams A W The clinical and pathological significance of gastric parietal cell antibody Ann N Y Acad Sci 1 4 657 1965

19 Irvine W J Stewart A G & Scarth L A clinical and immunological study of adrenocortical insufficiency (Addison's disease) Clin exp Immunol . 31 1967

20 Jackson W P U Steatorrhea and hypoparathyroidism Lancet 1 1086 1957

21 Kellen J Über Störungen des Kohlenhydratstoffwechsels bei erhöhter Tätigkeit der Schilddrüse Z ges inn Med 11 368 1956

22 — Stoffwechselstörungen im Kohlenhydrathaushalt bei Thyreotoxicose Z ges inn Med 11 80 1956

23 Kelley M L & Stewart J M Myxedema and intestinal malabsorption (nontropical sprue?) with severe hypomotility of the gastrointestinal tract Amer J dig Dis 9 79 1964

24 Kunin A S MacKay B R Burns S L & Halberstam M The syndrome of hypoparathyroidism,

adrenocortical insufficiency a possible sequel of hepatitis Amer J Med 34 856 1963
25 Lamberg B A Torstı P & Takkunen J The intravenous calcium infusion test (abstr) Acta endocr (Kbh) Suppl 119 31 1967
26 Morse W I Cochrane W A & Landrigan P L Familial hypoparathyroidism with pernicious anaemia steatorrhoea and adrenocortical insufficiency New England J Med 264 1021 1961
27 Salmi T & Lahesmaa P Pseudo-Hirschprungsche Erkrankung bei einem Myxodem Saugling Acta pædiat scand 45 428 1956
28 Salvesen H A & Bøe J Osteomalacia in sprue Acta med scand 146 290 1953
29 — Idiopathic hypoparathyroidism Acta endocrinol 16 214 1956
30 — Idiopathic hypoparathyroidism Lancet 1 1246 1957
31 Schiller K F R Spray G H Wangel A G & Wright R Hyperthyroidism and pernicious anaemia with special reference to gastric and intrinsic factor antibodies In Current topics in thyroid research (eds C Cassano and M Andreoli) p 795 Academic Press New York 1965
32 Siurala M, Julkunen H & Lamberg, B A Gastrointestinal tract in hyperthyroidism before and after treatment Scand J Gastroent 1 79 1966
33 Siurala M & Lamberg B A Stomach in thyrotoxicosis Acta med scand 165 181 1959
34 Taylor K B Roitt I M, Doniach D Couchman K G & Shapland C Autoimmune phenomena in pernicious anaemia gastric antibodies Brit med J 2 1347 1962
35 Tudhope G R & Wilson G M Deficiency of vitamin B_{12} in hypothyroidism Lancet 1 703 1962
36 Visakorpi J K & Gerber M Hypoparathyroidism with steatorrhoea and some features of pernicious anaemia in a 5 year-old girl Ann Pediat Fenn 9 178 1963
37 Williams M J Scott B Beck, J S & Blair J S Antigastric antibodies in hyperthyroidism Their relationship to impaired acid secretion Brit med J 1 388 1966
38 Williams E & Wood C The syndrome of hypoparathyroidism and steatorrhoea Arch Dis Childh 34 302 1959

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VENTRICULAR ARREST CAUSED BY THE VALSALVA MANEUVER IN A PATIENT WITH ADAMS STOKES ATTACKS ACCOMPANYING DEFECATION

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Abstract A report is given of a 66-year-old man presenting a history of syncopes accompanying defecation In this patient atrio-ventricular conduction disturbances and vagal cardiosensitivity were noted Performance of the Valsalva maneuver resembling straining at stool was associated with ventricular standstill and loss of consciousness This observation suggests that pathophysiological changes similar to those produced by the maneuver were responsible for the attacks of syncope It is postulated that myocardial hypoxia secondary to diminished blood pressure together with pronounced vagotonia were prime factors contributing to the asystole Ventricular arrest has not previously been described accompanying the Valsalva maneuver

Certain respiratory maneuvers and even normal respiration affect the rhythm of the heart but no severe arrhythmias or conduction disturbances have been described accompanying the Valsalva maneuver Syncopes accompanying the maneuver and similar procedures such as straining at stool and cough usually are caused by the resultant drop in blood pressure Below is presented a case showing that in a susceptible individual ventricular arrest induced by the maneuver may be the cause of syncope

CASE REPORT

The patient, a 66-year-old man was admitted on September 9 1964 In 1918 he suffered from severe influenza In 1940 he had duodenal ulcer For several years he had had low back pains In 1958 he went through surgery for papilloma of the urinary bladder Following a traffic accident in 1963 he complained of headaches however the syncopal seizures reported below occurred prior to this event.

For the last three years he suffered from fainting and loss of consciousness on exertion. In particular the attacks were induced by straining at stool and the patient would then assume the recumbent position as he had learned that his symptoms were alleviated by this technique

He was observed in the Department of Neurology in May 1964 and his symptoms were related to a possible intermittent insufficiency of the cerebral arteries Evidence of encephalopathia and cerebral atrophy were present Because of increasing frequency of the seizures he was later admitted to the local hospital (Innherred Sykehus Levanger Norway) where he had been observed previously and atrio ventricular block grade 1 had been revealed The patient now used isoprenaline tablets without any significant effect Twice during that hospital stay he fainted when he had bowel movements On one of these occasions he was observed by a nurse whose aid he had requested as he had felt uncomfortable and noted blurred vision When she arrived the patient lost consciousness completely he was pale and the breathing became stertorous He was pulseless for about fifteen seconds whereafter the pulse rate was 56/min irregular and the patient regained consciousness fairly soon An electrocardiogram obtained at this point was unchanged The episode described strengthened the suspicion that the attacks were of cardiac origin As the patient suffered severely from the disease he was admitted to this department for further cardiac evaluation and treatment

The patient complained of slight headaches and knee joint pains Between the attacks he did not appear ill but his general demeanor suggested some degree of cerebral arteriosclerosis On admission the pulse rate was 33/min regular B P 165/100 mm Hg A systolic murmur grade 2 was heard at the apex Otherwise the physical examination as well as routine urine and blood analyses were normal The ECG showed a grade 2 atrio-ventricular block 2 1 and right bundle branch block (Fig 1) Evaluation of the roentgenogram of the heart was unreliable since the patient had been unable to stand Right carotid sinus stimulation resulted in advanced atrio-ventricular block grade 2 changing the 2 1 block into 3 1 block Repeating of the procedure elicited transient atrio-ventricular block grade 3 and atrio-ventricular nodal rhythm Left carotid sinus stimulation gave rise to atrio ventricular nodal rhythm and numerous ectopic ventricular beats No characteristic clinical symptoms occurred in association with these procedures Rectal stim

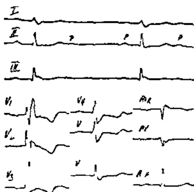


Fig 1 Admission electrocardiograms showing atrio ventricular block grade 2 with 2 1 block and right bundle branch block

ulation inserting the gloved finger induced neither electrocardiographic changes nor clinical symptoms The Valsalva maneuver performed with the patient in the supine position evoked no clinical or electrocardiographic changes When the Valsalva maneuver was carried out with the patient sitting, further widening and deformation of the QRS-complexes were noted whereupon high grade atrio-ventricular block occurred in which successive atrial impulses were blocked resulting in ventricular asystole for several seconds Some sinus acceleration took place simultaneously (Fig 2) The patient lost consciousness and the electrocard ographic recording had to be discontinued after a ventricular standstill of at least 4 6 sec The Valsalva maneuver with the patient sitting was repeated after some days Again high grade block was obtained resulting in a ventricular arrest of 3 5 sec but this time without concomitant syncope Later the patient identified the symptoms preceding the loss of

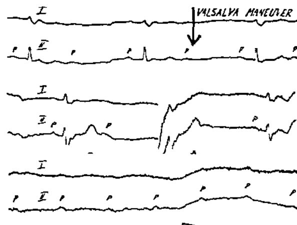


Fig 2 Electrocardiograms showing the effect of the Valsalva maneuver in the sitting position Onset of the maneuver indicated by arrow Consecutive recordings of standard Leads I and II demonstrating widening and deformation of the QRS-complexes high grade atrio-ventricular block with blocking of successive atrial impulses sinus acceleration and ventricular asystole

consciousness induced by the maneuver with his spontaneous seizures During the hospital stay he often complained of dizziness and attacks of faintness and once during the stay he experienced a short fainting spell accompanying defecation

On October 20 thoracotomy was performed and an artificial epicardial pacemaker applied The immediate result was satisfactory The heart rate was 75/min irregular as a result of parasystole (interference) Unfortunately the patient died suddenly and unexpectedly on the day after the operation Autopsy did not reveal any findings that could explain the sudden death Microscopic examination of the heart showed a subendocardial fibrous focus in the median wall of the right atrium

DISCUSSION

The cause of the sudden death in this patient remains unknown However ventricular fibrillation induced by the pacemaker may very well have been responsible since many predisposing factors were present parasystole early post operative period and parasympathetic tone (14) Constant supervision of such patients for some days after implantation of an artificial pacemaker seems important

Defecation and digital rectal dilatation as well may increase vagal tone and give rise to disturbances of the rhythm and conduction of the heart In one case ventricular fibrillation was induced by rectal stimulation (12) There is no indication however that reflexes of this type were involved in the mechanism underlying the attacks of syncope accompanying defecation in our patient

In the present case the Valsalva maneuver simulating straining at stool resulted in ventricular arrest and loss of consciousness This observation indicates that pathophysiological changes similar to those produced by the maneuver were responsible for the syncopal attacks The circulatory response to the Valsalva maneuver may be divided into four phases 1 the onset of straining 2 the period of sustained straining 3 the period immediately after the release of straining and 4 the subsequent period

The normal response to the maneuver is as follows During phase 1 a sudden increase in blood pressure occurs as straining empties blood from the lungs and the increased intrathoracic pressure is transmitted to the peripheral arterial vessels This results in a reflex slowing of the heart rate During phase 2 blood is prevented from entering the thoracic cage by the elevated intrathoracic pressure and as a result the cardiac

output falls and subsequently systemic blood pressure diminishes Produced by sympathetic stimulation there will be an increase in the heart rate In phase 3 with the sudden cessation of straining and loss of support of the intrathoracic pressure the blood pressure drops and the heart rate increases further In phase 4 there is an overshoot in blood pressure and reflex brady cardia (4) Some normal individuals (3) and pa tients with cardiac disease in particular (4 7) often fail to respond as outlined above In our patient blood pressure was not measured during the maneuver but the sinus acceleration observed during phase 2 suggests that there was a normal response to the maneuver

The development of the ventricular standstill during the Valsalva maneuver in this patient may be explained in the following ways

(*a*) *Myocardial hypoxia* Decreased blood pres sure resulted in diminished coronary blood flow thus producing myocardial hypoxia which has a direct depressant action on the atrio-ventricular node ((2) p 319) and ventricular pacemakers ((2) pp 523–526)

(*b*) *Vagotonia* Hypoxia increases the sensitivity of the vagal terminals of the heart in a constitu tionally vagosensitive patient ((2) p 526 (13)) In this patient pronounced vagal cardiosensitivity had been demonstrated and vagal stimulation in duced by the hypoxia was critical In addition the performance of the maneuver results in stim ulation of the pulmonary stretch receptors ini tiating a vagal reflex ((2) p 820)

(*c*) Following the asystole a further drop in blood pressure ensued thus giving rise to a vicious cycle

The fact that the atrio-ventricular block was more marked in the sitting than in the recumbent posture may be explained by diminished cardiac output sitting accentuating the fall in blood pres sure during the Valsalva maneuver Also para doxical postural mechanisms may have been pres ent (11) The cerebral symptoms secondary to the circulatory failure may have been precipitated more readily sitting due to the decreased hydro-static pressure in this position The symptomato-logy possibly also was influenced by the cerebral atrophy noted

Normal respiration and certain respiratory maneuvers affect the rhythm of the heart in various ways ((1) (2) p 820) but no severe ar rhythmias or conduction disturbances have been described accompanying the Valsalva maneuver ((2) p 820 (4 6 7 9 10)) However Bellet briefly mentions that cardiac arrest may be caused by the Valsalva maneuver following defe cation ((2) p 528) It is well established that the maneuver sometimes induces syncope due to the diminished blood pressure (3 8 10) which is believed to be the mechanism underlying the tussis syncope (5 10) The present case suggests that in some individuals ventricular arrest may be the cause of syncopes and even deaths follow ing cough

Vagal cardiosensitivity elicited by the carotid sinus reflex may possibly be used as a warning sign that sudden cardiac arrest or severe cardiac arrhythmias may occur in a susceptible individual under conditions that sensitize the vagal terminals in the heart ((2) p 524 (13)) Since vagal tone increases during the Valsalva maneuver similar procedures such as straining at stool and vigorous coughing should be prevented as far as possible in these patients by proper measures and instruc tions

REFERENCES

1 Adams C W Sinus arrest and syncope following full inspiration (respiratory syncope) Dis Chest 45 546 1964

2 Bellet S Clinical disorders of the *heart beat Henry* Kimpton London 1963

3 Booth R W Ryan J M Mellett H C Swiss E & Netti E *Hemodynamic changes associated with* the Valsalva maneuver in normal men and women J Lab clin Med 59 275 1962

4 Elisberg E I Heart rate response to the Valsalva maneuver as a test of circulatory integrity J Amer med Ass 186 200 1963

5 Hindberg, E & Schwartz, M Tussiv syncope Ugeskr Læg 124 1301 1967

6 Irvin C W Jr Valsalva maneuver as diagnostic aid J Amer med Ass 170 787 1959

7 Judson W E Hatcher J D & Wilkens R W Blood pressure responses to Valsalva maneuver in cardiac patients with and without congestive failure Circulation 11 889 1955

8 Klein L J Saltzman H A Heyman A & Sieker H O Syncope induced by the Valsalva maneuver Amer J Med 37 63 1964

9 Malmberg, R Albrecht, G Baltazaar A Bucking ham W B Levine H & Cugell D C The Val salva maneuver as a test of cardiac function in pa tients with pulmonary disease Amer Rev resp Dis 89 64 1964

10 Pedersen A Sandoe E Hvidberg E & Schwartz, M Studies on the mechanism of tussive syncop Acta med scand 179 653 1966
11 Schwartz, L S & Schwartz, S P The Adams-Stokes syndrome during normal sinus rhythm and transient heart block. Amer J Cardiol 12 505 1963
12 Scott R W & Sancetta S M Stokes-Adams attacks induced by rectal stimulation in a patient with complete heart block Circulation 2 886 1950
13 Sigler L H The cardioinhibitory carotid sinus reflex Amer J Cardiol 12 175 1963
14 Sowton E Artificial pacemaking and sinus rhythm Brit Heart J 27 311 1965

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NECROTIZING VASCULITIS WITHOUT VISCERAL INVOLVEMENT

Postmortem Examination of Three Cases with Affection of Skeletal Muscles and Peripheral Nerves

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Abstract The biopsy and postmortem finding are reported in thre elderly patients with a widespread ne rotizing vasculitis of the small arteries and arterioles of muscles and peripheral nerves and no clear involvement of visceral organs Most of the affected vesse s had a diameter between 50 and 200 μ Two of the cases also had a temporal arteritis and the third had a vasculitis of the small vessels surrounding the temporal artery All cases showed fibre loss in the peripheral nerves probably caused by ischaemic nerve lesions and widespread denervation atrophy of the muscles On the whole the nerves and muscles of the legs were most severely affected

All cases had attacks of fever high erythrocyte sedimentation rate and increased globulin fractions in the serum and all cases appeared to improve to some extent on steroid treatment The clini al picture had features in common with temporal arteritis and polymyalgia rheumat ca It is emphasized that elderly patients with such symptoms may develop signs of neuropathy during the course of the disease which is due to a vasculitis of small vessels This vasculitis may remain localized to muscles and peripheral nerves

The present report summarizes the biopsy and postmortem findings in three elderly patients with a widespread necrotizing vasculitis of unknown cause which affected the small arteries and arterioles of muscles and peripheral nerves and largely left the visceral organs intact Two of the patients also had a temporal arteritis The intervals between the onset of symptoms and death were from 1 to 3 years All cases received prolonged steroid treatment which may have modified the course of the disease and the findings at autopsy

The main purpose of the report is to emphasize the following points (*a*) In some cases of necrotizing angiitis there may be a selective affection of certain organs or systems such as muscles and peripheral nerves and little or no affection of visceral organs (*b*) Temporal arteritis in elderly patients may be accompanied by a widespread vasculitis of small vessels (*c*) There are intermediate types of necrotizing vasculitis which can not be classified into rigid subgroups

CASE REPORTS

Case 1

In 1961 a 60 year old man developed rheumatic pains which were vaguely referred to the finger joints shoulders and knees He received gold injections and physiotherapy and apparently improved somewhat over the following months In May 1964 he became worse with period of fever weight loss malaise and pains throu hout the body

He was admitted to hosp tal for one month in September–October 1964 On admiss on he had a temperature of 39 C severe and d ffuse pains and tenderness over the tendon attachments in the neck There were no s gns of polyarthritis The blood pressure was 1_0 65 The erythrocyte sedimentat on rate was 1 6 mm per hour White blood cell counts varied between 7400 and 11 00 per mm D fferential counts were normal Antistreptolysin and antistaphylolysin titres were within normal limits Repeated urinanalyses were unremarkable LE cell preparations were negative Total serum prote ns varied between 5 1 and 6 g per 100 ml Electrophoresis of serum showed decreased albumin content (36 aga nst normal 55–65) and increase in α globul n (9% aga nst normal 3–5%) α_2-globulin (19% against normal 4 8) and γ-globulin (35 against normal 16– 1)

Steroid treatment was started shortly after admission (30 mg prednisone daily) Marked subjecti e improvement followed and the temperature fell rap dly to normal levels

He was d scharged on steroid treatment and remained fairly well for –3 we ks He then noticed ncreasing pa ns in both feet and cal es and weakness of both legs The symptoms progressed over a few days and he was readmitted to hospital in No ember 1964



Fig 1 Inflammation in the wall of the temporal artery of case 1 Biopsy specimen Haematoxylin–eosin ×140

On the second admission he had a temperature of 39 C Both feet were almost paralytic with a severe hypoaesthesia up to the lower part of the calves The left leg was somewhat more affected than the right

After an increase of steroid doses to 60 mg daily the temperature again fell to normal levels and there was improvement in the general condition but no clear change in neurological symptoms

Two weeks after the second admission and some days after reduction in steroid doses to 50 mg he again developed fever and severe paresis and hypoaesthesia of the right arm and slight paresis also of the left hand There was some improvement after an increase of steroid doses to 100 mg daily and he was later largely afebrile However the pareses persisted and he also continued to have pains The erythrocyte sedimentation rate fell gradually during the course of the disease and stayed at 15 mm per hour during the last weeks He died from a severe bronchopneumonia on February 2 1965

Biopsies of the temporal artery and the soleus muscle

A biopsy specimen of the left temporal artery was taken in October 1964 (Fig 1) The lumen was nearly occluded by a thickened intima and the internal elastic membrane was fragmented There was a subacute inflammatory process in all layers of the artery with a heavy accumulation of lymphocytes and histiocytes and a fair number of neutrophilic leucocytes A platelet aggregate was attached to the intima No multinuclear giant cells were observed Two small vess ls (?00 μ) in the connective tissue around the temporal artery showed severe inflammatory reaction and recent thrombi in the lumen

A biopsy specimen of the left soleus muscle was taken in October 1964 three weeks after the onset of steroid therapy A marked inflammatory reaction was seen in the walls of several small arteries with a diameter about 200 μ (Fig 2) The lumen was occluded by fibrin thrombi The vessel walls were partly necrotic with patches of amorphous material which stained like fibrin The predominant inflammatory cells in and around the vessel walls were lymphocytes and histiocytes but there were also some neutrophilic leucocytes

Postmortem examination

The cause of death was a severe bilateral bronchopneumonia The large arteries showed a slight patchy atherosclerosis The arteries of the mesentery and other small visceral vessels were grossly unremarkable There was a small carcinoma with a diameter of 1 5 cm in the left kidney Otherwise the kidneys and other visceral organs were unremarkable No gross abnormalities were found in the brain or spinal cord

Microscopical sections were examined from visceral organs skin brain spinal cord and intraspinal nerve roots In addition multiple sections were examined from muscles and peripheral nerves These blocks were taken from the distal and proximal parts of the limbs and from the trunk

The sections from the skin brain spinal cord intraspinal nerve roots spleen heart liver kidneys and lungs showed no evidence of healed or active vasculitis Two small arteries in the peri adrenal adipose tissue and one artery between the muscle layers in the jejunum had thickened walls with a few lymphocytes in the media and adventitia These three vessels were the only ones which could indicate a vasculitis in the visceral organs

All examined muscles and nerves showed evidence of healed or slightly active vasculitis in arteries and arterioles (Fig 6) The affected vessels had a diameter between 25 and 400 μ the majority being from 50 to ?00 μ The vessel walls were greatly thickened with subintimal connective tissu proliferation patchy disruption of the internal elastic membrane and perivascular fibrosis Many vessels also showed a fairly marked intramural and perivascular accumulation of lymphocytes The lumen was often completely occluded presumably by organized thrombi, or there was evidence of recanalization Occasional perivenous lymphocytic aggregates were also found There was no evidence of acute vasculitis

The diseased nerve vessels were located both in the perineural connective tissue and within the nerve bundles Both sciatic nerves showed severe patchy nerve fibre loss some bundles were almost normal while neighbouring bundles contained very few fibres (Fig 3) In both tibial nerves only a few scattered myelinated fibres were left

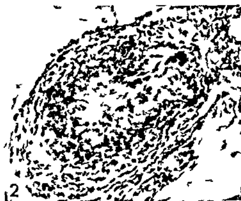


Fig 2 Inflammation and recent thrombus in a small muscle artery of case 1 Biopsy specimen Haematoxylin–eosin ×160

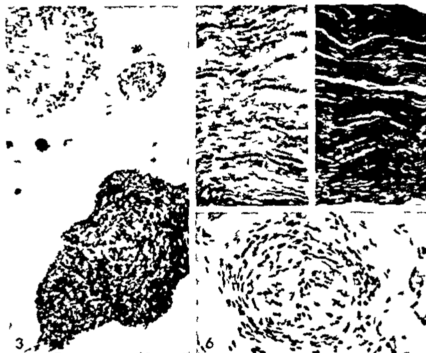


Fig. 3. Two small nerve bundles with nerve fibre loss (*top*) and adjacent normal bundle (*bottom*) in the right sciatic nerve of case 1. Woelcke's myelin stain. ×80.

Figs. 4 and 5. Abrupt transition from normal appearance (*5*) to Wallerian degeneration (*4*) in a nerve bundle from the left median nerve of case 1. Woelcke's myelin stain. ×80.

Fig. 6. Small muscle artery with healed vasculitis from case 1. The lumen is almost occluded by connective tissue and there is slight perivascular chronic inflammation. Haematoxylin-eosin. ×200.

In a longitudinal section of the left *median nerve* a normal fibre bundle showed abrupt transition into Wallerian degeneration in the distal part of the section (Figs. 4 and 5). It is therefore likely that the nerve fibre loss was due to ischaemic nerve lesions which were caused by occlusion of the small diseased *arteries in the nerve*.

All examined muscles showed varying degrees of denervation atrophy. This atrophy was most severe in the distal parts of the legs and relatively mild in the trunk muscles. No scars were found in the muscles after infarcts or other types of primary muscle destruction.

Several *medium-sized and large arteries* of the limbs and visceral organs were included in the sections. None of these showed any evidence of vasculitis.

Comment

This man, who died at the age of 64, had a history of severe "rheumatic" pains three years before death, which possibly was related to his terminal illness. Nine months before death he had diffuse pains throughout the body and fever. During the further course he had attacks of rapidly progressing asymmetrical pareses and hypoaesthesia in the legs and arms. The disease was accompanied by a strongly increased erythrocyte sedimentation rate, decreased serum albumin and increased serum globulins. There was a good clinical response to steroids, but relapses in spite of heavy doses. Although there were only vague local symptoms, a biopsy from the temporal artery showed a severe arteritis. A muscle biopsy showed an acute necrotizing vasculitis with thrombi in the small arteries and arterioles. Postmortem examination showed healed or slightly active vasculitis of the small arteries (50–200 μ) of all examined muscles and peripheral nerves. There was a severe patchy fibre loss in the peripheral nerves, probably due to ischaemic nerve lesions, and denervation atrophy of the muscles. The visceral organs showed little or no evidence of vasculitis.

Case 2

A previously healthy woman, who died at the age of 76, had pains in the back and period of fever one year before death. Later the pains spread to the neck, shoulders, arms and calves. She was admitted to hospital in December 1965, three months after onset of the disease. On admission she had a blood pressure of 130/90. During the first week she had irregular fever up to 39 C. Fourteen days after admission she awoke one morning with a large central scotoma of the right eye which later remained unchanged. She also complained of numbness and coldness of the feet, but no clear signs of neuropathy were found.

The erythrocyte sedimentation rate was 90 mm per hour. There was a slight normochromic anaemia. Leucocyte counts varied between 4700 and 19 600 per mm. Differential counts were normal. Repeated urinanalyses were negative. Total serum proteins were within normal limits. Electrophoresis of serum showed lowered albumin



Fig 7 Inflammation and recent thrombus in a small muscle artery from case . Biopsy specimen Haematoxylin–eosin × 30

(35) increased α globulin (1) slightly increased β globulin (18) and normal α and γ-globulins LE cell preparations were negative

Prednisone treatment (30 mg daily) was started on the day before the visual defect appeared Her temperature fell rapidly to normal levels and there was a clear improvement in her condition Later she had several new attacks of fever some of which o curred after attempts to reduce the steroid doses She was discharg d in a fairly good condition on steroid treatment Moderate pains in the shoulders and legs persisted

She was readmitted in July 1966 and stayed in hospital almost continuously until her death on October 3 1966 The second admission was caused by a fairly acute exacerbation of pains in the back and thighs Osteoporosis and compression fractures of several vertebrae were diagnosed These vertebral lesions were considered partly responsible for her new symptoms She was kept on steroid treatment and her symptoms remained largely unchanged until three weeks before death when she developed a pneumonia with septicaemia shock and oliguria She died from uraemia The oliguria was probably caused by an acute tubular nephropathy precipitated by the shock and septicaemia

Biopsies of the temporal artery and the m pectoralis major were taken three weeks after the first admission There were no local symptoms or signs of temporal arteritis (apart from the visual field defect) Histologically the temporal artery was unremarkable However several small arteries in the surrounding connective tissue showed vasculitis with necrosis of the walls recent fibrin thrombi and intense inflammatory reaction with lymphocytes and some neutrophilic leucocytes Similar changes were seen in several small vessels in the pectoralis muscle (Fig 7) The diameter of the affect d ve s ls was about 100 μ

Post mortem examination

Microscopic examination of the kidneys showed change which were consistent with a tubular nephropathy caused by shock There were no signs of vasculitis in th kidneys and the glomeruli were normal There was a moderate pulmonary oedema The heart was moderately hypertrophic and weighed 4 0 g Ther was a small ul u in the duodenum The aorta and other large arteries showed a moderate atherosclerosis The arteries of the mesentery and other visceral arteries were grossly normal There was a severe osteoporosis of the spine with compression fractures of several thoracic and lumbar vertebrae

Multiple blocks were taken for microscopic examination from many visceral organs brain spinal cord peripheral nerves and muscles No signs of vasculitis or other significant diseases were seen in the visceral organs or central nervous system Evidence of healed or slightly active vasculitis was found in numerous small arteries and arterioles in all examined mu cles and nerves (Fig 11) The diameter of the affected vessels was between 50 and 00 μ The lumen of the vessels was partly or completely occluded by connective tissue sometimes with evidence of re analization The internal elastic membrane showed patchy disruption or splitting and there was frequently a marked perivascular concentric fibro is Slight to moderate lymphocytic accumulat on was found in some of the vessels No vessels showed acute vasculit s

The affected vessels in the nerves were mainly located in the epi and perineurium but som were al o found within the nerve bundles Myelin stains showed a moderate patchy nerve fibre loss in the sciati nerves and a severe fibre loss in the tibial n rves (Fig 8) The fibre content of the median nerve was almost normal (Fig 9) None of the examined nerves showed recent myelin breakdown All examined muscles showed varying degrees of denervation atropy (Fig 10) The distal parts of the legs were most affected and the trunk muscles showed only slight atrophy There was no evidence of hea ed infarcts in the muscles

Comment

This 76 year old woman had a one year history of fever and pains throughout the body but most severely in the back and legs She had a strongly increased erythrocyte s dimentation rate decreased serum albumin and increased α globulins Three months after the onset of the disease she suddenly developed a large central scotoma of the right eye which later persisted There was a good clinical response to steroids but several recurrences during the course Biopsies from the temporal artery and the pectoralis muscle during the acute stage showed acute necrotizing vasculitis with recent thrombi in the small arteries and ar



Figs 8 and 9 Nerve fibre loss in the left tibial nerve (8) from case compared with a normal fibre content in the left median nerve (9) Woelcke's myelin stain ×80

Fig 10 Denervation atrophy of the left gastrocnemic muscle of case 2 Haematoxylin–eosin ×200

Fig 11 Completely occluded small vessel in the left sciatic nerve from case Haematoxylin–eosin × 00

terioles in the muscle and in the connective tissue around the temporal artery but no temporal arteritis On postmortem examination signs of healed vasculitis were found in numerous small arteries (50–200 μ) in the muscles and peripheral nerves but not in the visceral organs There was a quite marked nerve fibre loss in the nerves of the legs probably caused by ischaemic nerve lesions and widespread denervation atrophy of the muscles The distal muscles of the legs were most severely affected Although information about neurological symptoms is lacking the marked morphological changes in the nerves and muscles strongly suggest that the patient had a significant neuropathy particularly in the legs

Case 3

A 73 year-old man was admitted to hospital in 1965 with severe pain in the left side of the face and general malaise which had lasted for one week His face appeared swollen in the region of the left maxilla and there was some tenderness around the left temporomandibular joint The left temporal artery was thickened but not definitely tender The temperature was 38 C The erythrocyte sedimentation rate was 1 4 mm per hour Serum electrophoresis showed increased α_2 globulin (16%) LE cell preparations were negative

A *biopsy of the left temporal artery* showed a severe sub-acute inflammation with intramural accumulation of lymphocytes histiocytes and polymorphonuclear leucocytes (Fig 1) The internal elastic membrane was fragmented and there was a marked intimal connective tissue proliferation There was a recent thrombus in the lumen No multinuclear giant cells were seen Several small ar



Fig 12 Inflammation in the wall of the temporal artery from case 3 Biopsy specimen Haematoxylin–eosin ×120

teries and one little vein in the surrounding connective tissue showed acute necrotizing vasculitis with recent thrombi

Steroid therapy was started and he improved rapidly He was discharged on steroids (7 5 mg prednisone daily) and apparently did not have any recurrences No mention is made in the record about pain in other parts of the body or neurological symptoms

Four years before the onset of the temporal arteritis he had been admitted to hospital because of hypertension and a moderate proteinuria which in retrospect probably was due to a chronic glomerulonephritis He did not have any serious symptoms from his kidney disease until one year after the temporal arteritis when he developed a rapidly progressing uraemia and died after a few weeks illness

Post mortem examination

The cause of death was a chronic membranous glomerulonephritis There was no evidence of vasculitis in the kidneys Nor were there any signs of necrotizing glomerulitis as has been described in hypersensitivity angiitis (17) The heart weighed 570 g and showed myocardial fibrosis The large arteries showed severe atherosclerosis The brain and the spinal cord were grossly normal

Blocks were taken for microscopical examination from visceral organs muscles and peripheral nerves None of the visceral organs showed any evidence of vasculitis There was a severe hyaline arteriolosclerosis in the kidneys probably related to the hypertension

The sciatic and tibial nerves from both legs were examined Numerous small arteries and arterioles showed marked thickening of the walls with fibrosis and hyalinization of the media subtotal or complete occlusion of the lumen and perivascular fibrosis (Fig 14) The diameter of the vessels varied between 50 and 200 μ Most of them were found within the nerve bundles others were located in the perineurium No such changes were found in the nerves of the arms A few small arteries were also found in the muscles which showed fibrosis of the media and complete or subtotal occlusion of the lumen by connective tissue (Fig 13) In some of them the internal elastic membrane was disrupted None of the vessels in the nerves or muscles showed acute or chronic inflammation

A moderate nerve fibre loss was found in the sciatic and tibial nerves (Fig 15) while the nerves of the arms appeared normal All examined muscles from the legs showed quite marked denervation atrophy Slight denervation atrophy was found in the muscles from the arms and trunk

Since the changed vessels at autopsy in this case showed no inflammation it must be considered whether the thickened vessels in the nerves could be due either to a hypertensive arteriolosclerosis or to some kind of obliteration which was secondary to the nerve fibre loss However the normal vessels in the nerves from the arms would seem to preclude a generalized hypertensive arteriolosclerosis Furthermore the moderate nerve fibre loss could hardly cause the marked vascular changes It is therefore likely that the vascular changes represent the end stage of a vasculitis and that the nerve fibre loss also in this case was due to ischaemic nerve lesions

Comment

This 74 year old man had a history of histologically verified temporal arteritis one year before death Postmortem examination showed vascular changes in the nerves of both legs and a widespread denervation atrophy of the muscles which was most severe in the legs Although information about relevant clinical symptoms is



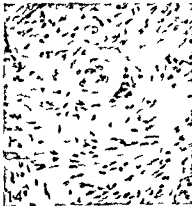
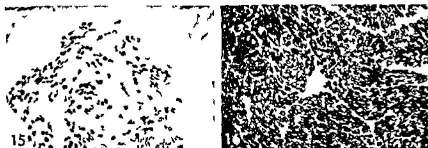


Fig 13 Subtotal occlusion by connective tissue and perivascular fibrosis of a muscle artery from case 3 Haematoxylin–eosin ×75

Fig 14 Hyalinization of the media and proliferation of connective tissue in a small artery in the left sciatic nerve from case 3 Haematoxylin–eosin ×200



Figs 15 and 16 Nerve fibre loss in the left tibial nerve (*15*) from case 3 compared with a normal fibre content of the left median nerve *16*) Haematoxylin–eosin × 100

lacking the morphological findings strongly suggest that also this patient had a vasculitis of the small vessels which had caused a widespread neuropathy

The cause of death was a chronic glomerulonephritis which had caused symptoms 4 years before the onset of the arteritis

DISCUSSION

From the biopsy and postmortem examinations it must be assumed that all three cases had a widespread necrotizing vasculitis which involved the small arteries and arterioles of muscles and peripheral nerves Most of the affected vessels had a diameter between 50 and 200 μ In addition cases 1 and 3 also had an arteritis of the temporal artery Case 2 had a vasculitis of the small vessels around the temporal artery but no temporal arteritis

Generalized necrotizing vasculitis of small vessels is known mainly to affect visceral organs although simultaneous involvement of nerves and muscles are frequent The vasculitis of the present cases was remarkable in the lack of involvement of visceral organs The only vague indication of visceral affection was seen in case 1 which showed a few thickened arteries in the peri adrenal adipose tissue and in the intestine These peri adrenal vessels were close to nerve bundles and may in fact represent nerve affection Necrotizing vasculitis may thus show a selective affinity for certain organs or systems A similar case in which only the peripheral nerves were involved was described as polyarteritis nodosa by Kernohan and Woltman (9)

All three cases showed a clear nerve fibre loss in the peripheral nerves probably caused by occlusion of the small diseased vessels in the nerves Varying degrees of denervation atrophy were present in most examined muscles in all cases This indicates a generalized nerve affection although there were marked quantitative differences in the various areas Since the small vessels within the nerve bundles were affected the lesions sometimes involved only small parts of the cross section of the nerves (Fig 3) Generally the distal parts of the limbs were most severely affected as could be expected from a summation of multiple small lesions along the course of the nerves

Case 1 had several attacks of rapidly progressing asymmetrical paresis and hypoaesthesia of the limbs Information about neuropathy is lacking in the two other cases However the muscle atrophy and the nerve lesions of the legs were so severe that overt clinical symptoms probably were present also in these cases

Although both muscles and nerves were affected by the vasculitis only the nerves showed evidence of ischaemic lesions This may be due both to a better collateral circulation and to a greater tolerance to ischaemia in the muscles

It is remarkable that in all cases the vascular lesions found at autopsy were either completely healed or showed only slight chronic inflammation All cases received prolonged treatment with steroids and it is possible that this in part may be responsible for the uniform histological picture as has been claimed in steroid treated cases of polyarteritis nodosa (2 20 21) It should be noted however that relapses occurred even on very high steroid doses (80–100 mg daily)

Both clinically and pathologically the cases have features in common with several of the previously reported subgroups of necrotizing vasculitis However they seem to represent intermediate typ s and do not quite fit into anyone particular group Four relevant groups of disorders will be discussed briefly in this respect 1 Polyarteritis nodosa proper 2 hypersensitivity

angiitis 3 temporal arteritis (giant cell arteritis) and 4 polymyalgia rheumatica which according to some authors may be caused by a giant cell arteritis (1 3 4 6 13 15 16 19 22)

It is not yet clear whether polyarteritis nodosa and hypersensitivity angiitis are entirely separate disorders (14 17 18) However in the typical case polyarteritis nodosa affects large and medium sized arteries and healed and recent vascular lesions are usually found in the same case (12 17 23) Hypersensitivity angiitis was described by Pearl Zeek (12 23) as a fulminating disease with a necrotizing vasculitis of small visceral and other vessels particularly those of the lungs Allergic or hypersensitive mechanisms have been implied but rarely convincingly proved Cases with a more protracted course have later been included in this group (17) In contrast to polyarteritis nodosa the vascular lesions of hypersensitivity angiitis all show the same stage of development (17) The small vessel disease of the present cases may resemble that of hypersensitivity angiitis However the lack of visceral involvement the intermittent clinical course and the affection of the temporal arteries are atypical features of this disorder

The histological changes in the temporal arteries of cases 1 and 3 resemble those seen in the clinical syndrome temporal arteritis with the exception that no multinuclear giant cells were observed However it is not likely that the giant cells are of specific importance for this disease The giant cells probably represent some kind of foreign body reaction to damaged elastic fibres (7 10) and it is doubtful whether further pathogenetic significance should be attached to them Several cases of otherwise typical temporal arteritis have been described without giant cells (7 11 16) and giant cell reaction has occasionally been observed also in polyarteritis nodosa (5 7)

Many cases with active temporal arteritis apparently lack or show only vague local temporal symptoms and may present under the clinical picture of polymyalgia rheumatica (1 3 4 6 13 15 16 19 22) Some of these cases have shown active vasculitis also in large muscle arteries (8) Thus there may apparently be transitions between the clinical syndromes temporal arteritis and polymyalgia rheumatica

The clinical picture in our case 3 was fairly typical of a temporal arteritis Case 1 had only vague local temporal symptoms in spite of an active arteritis and the clinical picture resembled that of polymyalgia rheumatica for several months before the pareses appeared Case 2 which had no temporal arteritis but a vasculitis of the small vessels surrounding the artery also had many clinical features in common with polymyalgia rheumatica The central scotoma in this case suggests that the vessels of the eye or optic nerve were also affected

Widespread vasculitis of small vessels with affection of peripheral nerves as was seen in our cases has not been described either in temporal arteritis or polymyalgia rheumatica It should be noted however that the neuropathy was recognized clinically only in one case There is also a considerable chance that a small muscle biopsy may be negative even in widespread vasculitis Further detailed clinical and postmortem studies are therefore needed in cases of polymyalgia rheumatica and temporal arteritis to investigate the possible relationship to the type of vasculitis described here

It is apparent from these considerations that our cases do not fit into previously described groups of vasculitis and exact classification of the cases is at present not possible Regardless of this it should be emphasized that elderly patients with clinical pictures resembling temporal arteritis or polymyalgia rheumatica may develop signs of neuropathy during the course of the disease which is caused by a vasculitis of the small endoneural vessels The vasculitis of these cases may remain localized to muscles and peripheral nerves and leave the visceral organs intact

REFERENCES

1 Alestig K & Barr J Giant cell arteritis A biopsy study of polymyalgia rheumatica including one case of Takayasu s disease Lancet 1 1228 1963
2 Baggenstoss A H Shick R M & Polley H F The effect of cortisone on the lesions of periarteritis nodosa Amer J Path 27 537 1951
3 Bruk M I Articular and vascular manifestations of polymyalgia rheumatica Ann rheum Dis 26 103 1967
4 Dixon A St J Beardwell C Kay A Wanka J & Wong Y T Polymyalgia rheumatica and temporal arteritis Ann rheum Dis 25 203 1966
5 Gordon L Z & Thurber D C Temporal arteritis Report of a case and a comparison with respect to periarteritis nodosa Arch Path 42 402 1946

6 Hamrin B Jonsson N & Landberg, T Involvement of large vessels in polymyalgia arteritica Lancet 1 1193 1965
7 Heptinstall R H Porter K A & Barkley H Giant cell (temporal) arteritis J Path Bact 67 507 1954
8 Hudson R E B Cardiovascular pathology vol I Edward Arnold London 1965
9 Kernohan J W & Woltman H W Periarteritis nodosa A clinicopathologic study with special reference to the nervous system Arch Neurol Psychiat (Chic) 39 655 1938
10 Kimmelstiel P Gilmour M T & Hodges H H Degeneration of elastic fibers in granulomatous giant cell arteritis (temporal arteritis) Arch Path 54 157 1952
11 Knorring J von Erma M & Lindstrom B The clinical manifestations of temporal arteritis Acta med scand 179 691 1966
12 Knowles H C Zeek P M & Blankenhorn M A Studies on necrotizing angiitis IV Periarteritis nodosa and hypersensitivity angiitis Arch intern Med 9 789 1953
13 Kogstad O Polymyalgia rheumatica — arteritis temporalis T norske Lægeforen 83 534 1963
14 McCombs R P Systemic allergic" vasculitis Clinical and pathological relationships J Amer med Ass 194 1059 1965
15 Olhagen B Polymyalgia rheumatica A form of senile arteritis? Acta rheum scand 9 157 1963
16 Paulley J W & Hughes J P Giant-cell arteritis or arteritis of the aged Brit med J 2 1562 1966
17 Reidbord H E, McCormack L J & O Duffy J D Necrotizing angiitis II Findings at autopsy in twenty seven cases Cleveland Clin Quart 3 191 1965
18 Rose G A & Spencer H Polyarteritis nodosa Quart J Med 26 43 1957
19 Russel R W R Muscular involvement in giant cell arteritis Ann rheum Dis 21 171 1962
20 Shick R M Baggenstoss A H Fuller B F & Polley H F Effects of cortisone and ACTH on periarteritis nodosa and cranial arteritis Proc Mayo Clin 25 492 1950
21 Symmers W St C Pathological findings in cases of polyarteritis nodosa after treatment with adrenocorticotropic hormone J Path Bact 66 109 1953
22 Wilske K R & Healey L A Polymyalgia rheumatica A manifestation of systemic giant-cell arteritis Ann intern Med 66 77 1967
23 Zeek P M Smith, C C & Weeter J C Studies on periarteritis nodosa III The differentiation between vascular lesions of periarteritis nodosa and of hypersensitivity Amer J Path 24 889 1948

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LOW MOLECULAR DEXTRAN IN CHRONIC CIRCULATORY FAILURE

Effect Estimated by Lung Diffusing Capacity

P Sølvsteen[1] and Eva Nathan

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Abstract By means of the steady state method 11 determinations of the lung diffusing capacity (D_L) were made in ten patients suffering from chronic pulmonary diseases before and after infusion of 500 ml of 10% low molecular weight dextran (Rheomacrodex®)

D_L did not change after the infusion This challenges the assumption that low molecular weight dextran exerts a dilating effect on the lung capillaries and it also indicates either that no aggregation of erythrocytes occurs or that any aggregation is not inhibited by low molecular dextran

On the basis of the present studies and of those by other authors the question is discussed as to whether low molecular weight dextran might be supposed to improve the supply of oxygen to tissues with threatening gangraena caused by arteriosclerosis better than do other plasma expanders This does not appear to be immediately probable

It is emphasized that the investigations do not give any information as to the effect of low molecular weight dextran in acute circulatory disorders such as shock severe trauma myocardial infarction etc in which cases a pronounced aggregation of erythrocytes inter alia might exert an essential influence on the course of the disease

During recent years low molecular weight dextran (Rheomacrodex®) has been used in the treatment of arteriosclerotic vascular diseases with threatening gangraena (4 8 10) It was supposed first that by reducing the viscosity of the blood low molecular weight dextran would increase the blood circulation in the hazardous region and secondly that it prevented aggregation of the erythrocytes whereby the surface was increased through which oxygen could diffuse from the blood to the tissues

A method to show whether an improved supply of oxygen to such small areas of tissue is actually obtained is difficult to establish However if such an effect is present in the peripheral vessels a similar effect resulting in an increased oxygen uptake must be expected to occur in the lung vessels not least in patients with chronic lung diseases in whom pulmonary circulation is already poor

This is of interest firstly because it is possible to make determination covering a fairly large capillary network in the pulmonary circulation which may contribute to clarifying the effect on the peripheral circulation and secondly because such an effect might be of practical value in the treatment of patients with chronic lung diseases

As will be discussed later all possible effects of low molecular weight dextran will tend to increase the lung diffusing capacity (D_L) By measuring D_L it is therefore possible to examine whether low molecular weight dextran has the above mentioned effects on the pulmonary circulation

The object of the present study was to investigate whether infusions of low molecular weight dextran change the conditions of circulation in the lungs in patients suffering from chronic pulmonary diseases and on the basis of the results obtained to discuss the effect of low molecular weight dextran in patients with peripheral vascular disorders

METHODS

By the lung diffusing capacity for carbon monoxide is meant the volume of carbon monoxide (in ml) which is taken up per minute over the entire lung surface when the difference between the carbon monoxide tension in the alveoles and the blood is 1 mm Hg

D was determined by employing the method of Bates et al (?) slightly modified Instead of applying a "bag-in-the-box" principle and determining the respiratory

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Table I *Data of subjects and lung diffusing capacity before (D_{L-1}) and after (D_{L-2}) low molecular dextran*

Subject	Sex	Age	Diagnosis	D_{L-1} (ml min^{-1} mm Hg^{-1})	D_{L-2}
C J	♂	59	B E	9 4	8 8
H S	♂	75	B	9 4	8 2
—	—	—	—	7 9	8 5
H H	♂	80	B	8 5	8 9
A M	♀	69	A B E C	4 8	4 5
A K	♂	64	B A C	11 3	11 0
A S	♀	56	B	9 5	9 9
K B	♂	52	B E	7 1	7 3
K L	♀	68	A B	10 7	9 6
P L	♂	22	A	12 1	10 3
E J	♀	49	A B E	11 1	10 0
Mean				9 3	8 8

Symbols A = asthma bronchiale E = emphysema pulm B = bronchitis chronica C = cor pulmonale

volume by means of a spirometer connected to the box the expired air was collected in a rubber bag and at the termination of the experiment the volume in the bag was determined by forcing the air through a gasmeter The concentration of carbon monoxide in the air mixture expired in the inspired air and in the end expiratory air was measured by means of a Beckman infra red carbon monoxide analyzer

In connexion with each examination duplicate determinations were carried out The interval between the two determinations was 10 to 15 minutes

Eleven determinations were made in a total of ten patients In eight of these cases an intravenous infusion of 500 ml of 10 low molecular weight dextran was given shortly after the initial determination of D_L In three cases the dextran was not given until the day after the initial determination of D_L The 500 ml of 10 low molecular weight dextran were infused over a period of 1 / to 2 hours The determination of the D_L was made between 30 minutes and 1¹/ hours after the infusion

Hence a period of not less than 3 hours passed between two duplicate determinations of D_L By inserting reasonable volumes of carbon monoxide taken up during the experiments and cleared during the interval between these experiments on the basis of Linderholm's equation (6) *it can be calculated that the carbon* monoxide present in the blood will only cause the D_L determination to be between 2 and 3 lower in the second session than in the first

RESULTS

Table I presents the results of determinations of D_L before and after infusion of low molecular weight dextran In addition the age and sex of the patients and the diagnoses are stated

The table shows that after infusion of low molecular weight dextran an average fall in D_L of 5% occurred This decrease is not statistically significant ($p > 0 1$) and furthermore as previously mentioned a fall of 2–3% must be expected when repeated measurements are made on the same day

Therefore the conclusion from the determinations is that the D_L was not changed after infusion of low molecular weight dextran and more particularly it was not increased

DISCUSSION

From the alveoles carbon monoxide must diffuse through the alveolar walls the capillary walls the blood plasma the erythrocyte membrane and the erythrocyte plasma whereupon it is bound chemically to the haemoglobin

Therefore the D_L depends on the nature of the alveolar walls (thickness content of fibrous tissue and other physiochemical properties) on the number of functioning capillaries in contact with functioning alveoles and on the content of haemoglobin in the lung capillaries (13) Furthermore the D_L is dependent on the total erythrocyte surface in the lung capillaries On the other hand if no extreme changes occur such as stagnation of the blood so that the carbon monoxide tension of the blood cannot be considered to be zero the D_L does not depend on the magnitude of the perfusion (11 12) Hence by determining the D_L information is obtained as to whether a certain plasma expander (in the present case low molecular weight dextran) influences solely the perfusion or whether it has some other of the effects mentioned above

In small vessels low velocities will produce a tendency to aggregation of the erythrocytes and consequently a tendency to a high viscosity of the blood From a theoretical point of view this will create a tendency to non uniform distribution of the blood in the capillaries (3) which can be illustrated as follows

If the blood had to pass through all capillaries the rate of velocity would perhaps be so low and consequently the aggregation of erythrocytes so pronounced that the circulation would stop completely On the contrary if the blood were to pass through fewer vessels a suitably high rate of velocity without any aggregation of erythrocytes worth mentioning could be maintained In

the case of an uneven distribution of the perfusion of this nature reduced blood viscosity or increased perfusion in the afferent vessels would either give rise to an increased velocity in the existing vessels or to dilatation of new vessels

As is the case when other plasma expanders are infused infusion of low molecular weight dextran will increase the filling pressure of the heart and thereby the cardiac output (5) The perfusion through the lungs must therefore be increased Furthermore low molecular weight dextran is supposed to produce a decrease in the blood viscosity and thereby to contribute to a greater extent to the opening of new vessels

However the investigations show that the D_L is not increased by infusion of low molecular weight dextran Therefore the content of erythrocytes in the lung capillaries cannot be increased and this opposes the assumption that the lung capillaries are considerably dilated

The absence of an increase in D_L also indicates either that no important aggregation of erythrocytes is present in the pulmonary circulation or that any existing aggregation is not counteracted by administration of low molecular weight dextran Disaggregation of the erythrocytes per se would enlarge the surface of the erythrocytes and thereby increase the D_L

Hence the effect of an infusion of low molecular weight dextran in patients suffering from chronic pulmonary diseases does not seem to differ much from that observed in healthy persons after infusions of albumin solutions Also in these cases an increase in cardiac output is seen not associated with an increase in the D_L (12)

The results of the present investigations are in accord with tho e reported by Korsgren (5) obtained from studies of healthy persons and patients suffering from mitral disorders His examinations showed that infusions of low molecular weight dextran did not impair the pulmonary resistance or increase the blood volume of the lungs However the blood volume measured by applying the Korsgren method comprised a considerably greater volume than that of the pulmonary capillaries including the entire volume of blood found between the pulmonary artery and the left atrium Thus when th Korsgren m thod is employed the possibility cannot be discount d that the blood is displaced from one part of the lung vessels to another during the infusion Since the infusion resulted in an increase in pressure in the pulmonary artery it might be supposed that the blood was displaced from the arteries to the capillaries or the veins The investigations reported in the present paper show that the D_L does not increase Therefore any displacement of the blood cannot be to the capillaries

Consideration must be given as to whether the absence of an increase in the D_L subsequent to infusion of low molecular weight dextran might be caused by the fact that prior to the infusion the dilatation of all the capillaries was maximum However in patients suffering from chronic bronchitis and emphysema the D_L increases during exercise (1 7 9) This increase is more pronounced than that which might be caused solely by an increase in ventilation (1) and this indicates that it is caused by an increase in the capillary blood volume in the lungs Korsgren (5) found that an infusion of low molecular weight dextran succeeded by infusion of acetylcholine increased the blood volume of the lungs although low molecular weight dextran alone did not exert this effect Therefore it does not appear probable that the capillaries were incapable of dilating

Hence the results do not give any reason for supposing that infusion of low molecular weight dextran increases the surface through which carbon monoxide (and oxygen) diffuses or that it has any effects on the pulmonary circulation in patients suffering from chronic lung disorders other than those exerted by other plasma expanders

As regards the peripheral circulation Korsgren showed also that the resistance is reduced after infusion of low molecular weight dextran or other plasma expanders Hence in the peripheral circulation plasma expanders must cause a vasodilatation but it is uncertain in which part of the circulation such dilatation occurs As previously mentioned in order that low molecular weight dextran may produce an increase in the diffusion surface a disaggregation of the erythrocytes must be obtained resulting in an increase in the erythrocyte surface and possibly also in a dilatation of the capillaries Although the mechanisms governing the regulation of the peripheral and the pulmonary circulation doubtless differ widely it appears most unlikely that low molecular weight dextran as far as disaggregation of the erythrocytes is concerned should have dif

ferent effects in the peripheral and the pulmonary circulations To this should be added that in tissues with incipient ischaemia the local hypoxia in itself presumably has produced maximum dilatation of the capillaries before the infusion is started

Consequently the results of the investigations do not provide any basis for supposing that infusion of low molecular dextran will result in a better supply of oxygen to peripheral tissues e g to tissues with threatening gangraena because of arteriosclerosis than is the case when other plasma expanders are infused

It should be emphasized that the investigations do not give any information as to the effect of low molecular weight dextran in acute circulatory disorders such as shock severe traumatic tissue injuries myocardial infarctions etc in which cases a pronounced aggregation of erythrocytes inter alia might exert an essential influence on the course of the disease

ACKNOWLEDGEMENTS

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REFERENCES

1 Apthorp G H & Marshall R J clin Invest 40 1775 1961
2 Bates D V Bouot N G & Dormer A E J Physiol (London) 129 237 1955
3 Burns A M Lacoste J & Lockhart A Communication at Entretiens de physio pathologie respiratoire Nancy 1967
4 Bergan J J Trippel O H Kaupp H A Kukral J C & Nowlin W F Arch Surg 91 338 1965
5 Korsgren M Effect of changes of total blood volume on pulmonary blood volume Conference on pulmonary circulation p 115 Universitetsforlaget Oslo 1965
6 Linderholm H Acta med scand 156 413 1957
7 — Acta med scand 163 61 1959
8 Losel H Med Klin 59 1433 1964
9 McNamara, J Prime F J & Sinclair J D Thorax 14 166 1959
10 Powley P H Amer Heart J 67 424 1964
11 Rosenberg E & Forster R E J appl Physiol 15 883 1960
12 Ross J C Frayser R & Hickam J B J clin Invest 38 916 1959
13 Sølvsteen P Lungediffusionskapaciteten med særligt henblik på bestemmelsen ved ujævn ventilation p 26 Thesis Munksgårds forlag, Copenhagen 1966

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DIABETIC COMA WITHOUT KETOACIDOSIS

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Abst act As early as the 19th century diabetic coma without ketoacidosis (d c w k) was considered by some uthors to be a disease differing from the common dia betic coma During recent years more than 60 cases have been published in the literature still carrying a mortality of 50

We have collected another five cases None of the pa t ents was in deep coma and only one died Four of the patients had previously been treated with thiazides which in some of the cases might have indu ed the diabetes

The mechanism of the development and the treatment of d c w k is discussed Administration of insulin and large quantities of fluid parenterally is necessary if the high mortality is to be reduced For rehydration purposes we prefer hypotonic saline containing 100 mEq NaCl per litre

In a paper concerning diabetic coma associated with renal disease Warburg (26) in 1925 presented a survey of the history of the diabetic coma It appears from this survey that in the middle of the 19th century it was supposed that the dete rioration of consciousness in comatose patients was the result of acetone intoxication As early as in the 1880 s however it was realized that not all patients suffering from diabetic coma had acetonuria In addition to the classical type of coma associated with acetonuria and deep breath ing described by Kussmaul and others Frerichs (8) and Dreschfeld (7) presented another type characterized by hyperglycaemia and sudden col lapse or cardiac failure" accompanied by weak ness cold extremities weak pulse drowsiness and atypical Kussmaul breathing but without acetone in the expired air or the urine Death occurred within 10 to 20 hours

Similar cases have been described by Joslin (15) and Rosenbloom (20)

Warburg s material comprised four patients suffering from diabetic coma without acetonuria or with only slight acetonuria

These four patients were suffering from renal disease and Warburg was inclined to believe that for this reason the ketones present in the blood could not be excreted with the urine Con sequently Warburg supposed that ketonaemia was present

In three of the patients this assumption might be correct since in these patients a smell of acetone and increased urinary excretion of am monia were observed On the other hand it was doubtful whether the fourth patient had acidosis and ketonaemia This patient was a 57 year old man who had previously been in good health He was admitted to hospital deeply comatose his blood sugar on admission was 670 mg per 100 ml He was dehydrated occasionally with deep breathing which however was not typical (i e of Kussmaul type) The expired air did not smell of acetone no acetonuria was present and the urin ary excretion of ammonia was not increased The patient was treated with insulin and saline infu sions His condition improved rapidly and he was discharged from hospital initially without insulin but later such treatment had to be in stituted

Thus it has been observed a long time ago that diabetic coma may occur without being associated with acetonuria but the classification of such cases as an independent type of diabetic coma was not maintained Interest was revived when in 1957 de Graef and Lips (6) and Sament and Schwartz (23) published two cases of diabetic coma without ketoacidosis (d c w k) Since that time more than 60 cases have been described (14 18) The condition is still considered to be rare although the diagnosis is made with increas ing frequency and the disease seems to be more

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common than formerly supposed The lethality is high This is apparent from a survey of the literature over the last decade which shows that 28 out of 53 patients have died (18)

During the last four years five patients have been admitted to the medical departments B and F of the Frederiksborg County Hospital under the diagnosis of d c w k However it should be stated that these patients were rather precomatose than actually comatose The case reports of these patients are given below with the object of drawing attention to the frequency and serious prognosis of this entity and to the varying concepts in respect to the pathogenesis and treatment Three of these cases have been reported previously by Grønbæk and Kjeldsen (10) with the object of illustrating the diabetogenic effect of thiazides

CASE REPORTS

1 *Case B 554/63–64*

A 69 year-old extremely obese female One of her cousins had diabetes mellitus For a period of four years the patient had been treated with chlorothiazide because of heart failure and during the last five weeks she had also received digitalis

For a couple of weeks the patient had vomited and had diarrhoea and after that time she had complained of increasing thirst and polyuria She was admitted on April 25 1963 On admission she was soporous and dehydrated Her blood sugar was 932 mg per 100 ml No acetonuria or polypnoea was present Standard bicarbonate was 29 5 mEq/l, serum creatinine 2 2 mg/100 ml ECG revealed left axis deviation and Cohn effect

During the first 24 hours the patient was given 180 I U of insulin and 4300 ml of fluid half of which was given parenterally as a hypotonic electrolyte solution (see later) Subsequently the blood sugar was 273 mg/100 ml and the patient was completely alert The serum creatinine became normal within 14 days Since that time the patient s diabetes has been stabilized on diet alone although treatment with chlorothiazide and digitalis was still required because of the cardiac insufficiency

2 *Case F 240/60–61*

A 72 year-old moderately obese female No family history of diabetes mellitus Treated over a period of two years with thiazide and digitalis because of cardiac failure Furthermore the patient received chlorpromazine and used considerable amounts of analgetics

After three weeks increasing thirst, polyuria, fatigue and vomiting, the patient was admitted to hospital on December 14 1963 She was slightly dehydrated and drowsy The blood-sugar was stated to be higher than 387 mg/100 ml and two hours after administration of 40 I U of insulin it was 726 mg/100 ml. No acetonuria was present The standard bicarbonate was 16 4 mEq/l and serum creatinine 3 6 mg/100 ml,

During the first 24 hours the patient received 128 I U *of insulin and 2000 ml of* isotonic electrolyte solution parenterally and also large amounts of fluid orally Subsequently the blood sugar fell to 296 mg/100 ml

On discharge the patient still received 20 I U of insulin daily The diuretics had been withdrawn

It should be stated that as early as 1961 slight renal failure associated with a serum creatinine value of 1 6 mg/100 ml had been observed Furthermore at subsequent follow up examinations the serum creatinine was found to be increased (up to 6 0 mg/100 ml) and the patient had often had low standard bicarbonate (as low as 12 mEq/l) without any acetonuria

3 *Case F 761/64–65*

A 59 year-old very obese female No family history of diabetes mellitus In 1942 the patient underwent strumectomy because of exophthalmic goitre For a period of about two years the patient had had a tendency to oedema and slight hypertension for which she received chlorothiazide Furthermore she was treated with thi ethylperazin maleate (Torecan®) phenyl butazone (Butazolidin®) 200 mg daily chlorprothixene (Truxal®) 45 mg daily rescinnamine (Rescamin®) 1.5 mg daily

During the previous month the patient had complained of thirst loss in weight and impairment of vision Furthermore she had had loose stools for the previous two weeks

She was admitted to the hospital on August 8 1964 During the preceding 24 hours she had had nausea and vomiting and had been drowsy The temperature was 39 C the blood-sugar 1218 mg/100 ml There was no acetone in the urine The standard bicarbonate was 23 3 mEq/l At the clinical examination the patient was dehydrated The serum creatinine was 3 3 mg/100 ml and the serum protein 9 4 g/100 ml

During the first 24 hours 440 I U of insulin and 1850 ml of fluid were given mainly as infusions of hypotonic electrolyte solutions The blood sugar was reduced to 570 mg per 100 ml The patient was then completely alert, the temperature normal but anuria was present During the following 24 hours the patient received 304 I U of insulin and 4750 ml of fluid whereupon the diuresis rose to normal values The blood sugar and serum creatinine became normal

On discharge the patient s diabetes was treated with diet Insulin was not required The blood pressure ranged around 200/100 mg Hg It was not found necessary to continue the chlorothiazide treatment

4 *Case F 621/64–65*

A 54-year-old slightly obese female No known family history of diabetes mellitus The patient had suffered from diabetes mellitus for ten years For some time the patient had been treated with diet and oral hypoglycemic agents but later she stopped taking the drug and it is doubtful whether she has kept her dietary schedule

For many years the patient had suffered from gastric dyspepsia The symptoms became more pronounced and on August 13 1964 she was admitted to hospital because of voluminous acid aqueous vomiting and increasing drowsiness On admission she was extremely de

hydrated The blood sugar was 881 mg/100 ml there was no acetone in the urine The standard bicarbonate was 41 9 mEq/l serum creatinine 9 1 mg/100 ml serum potassium 2 9 mEq/l serum chlorides 47 2 mEq/l and serum sodium 140 mEq/l

During the first 12 hours the patient received 250 I U of insulin and 6850 ml of fluid first as infusions of hypotonic solutions later—when the above electrolyte analyses were available—as infusions of potassium and sodium chloride Because of falling blood pressure serum and blood were al o given

The blood sugar fell to 99 mg per 100 ml and the patient regained complete consciousness During the night she suddenly aspired large volumes of stomach contents The patient died in spite of tracheo-bronchial suction respirator treatment blood transfusions and subsequent cooling because of severe hyperthermia

Autopsy revealed severe pulmonary oedema a large fistula between the gall bladder and the duodenum and slight gastritis

5 *Case F 1051/66–67*

A 81 year-old slightly obese female No known family history of diabetes mellitus Over a period of several years the patient had been treated with digitalis and a thiazide diuretic because of cardiac failure Furthermore she was given tolbutamide (Rastinon®) 500+250 mg daily because of slight diabetes

She was admitted to hospital on August 3 1966 after having been dizzy drowsy and feverish for a few days The temperature was 40 C the blood-sugar 607 mg/100 ml There was no acetone in the urine The standard bicarbonate was ?3 8 mEq/l the serum creatinine 3 1 mg/100 ml During the first ?4 hours the patient was given 240 I U of insulin and 7100 ml of fluid mainly as infusions of hypotonic electrolyte solutions

The next day the blood sugar was ?18 mg/100 ml During the following 4 hours another 168 I U of insulin and 4200 ml of fluid were given orally and parenterally Although no focus of infection had been found the patient was treated with penicillin The temperature became normal two days after admission

Later the patient developed severe urinary infection and urinary retention, and an indwelling catheter was introduced In addition a large bed-sore developed in the sacral region

The patient was transferred to a residential home where she died two months later She was treated throughout with Insulin Retard® 12 I U a m and 8 I U p m

DISCUSSION

The five cases of d c w k reported were all seen within a period of four years During the same period twenty five patients suffering from classical diabetic coma or precoma associated with keto acidosis had been admitted The non ketotic coma consequently does not appear to be extremely rare as previously supposed

Various hypotheses have been advanced as to why some patients with diabetic coma do not develop ketoacidosis

Many of the cases described in the literature occurred in connexion with the administration of thiazides Therefore Schwab et al (24) supposed that the mechanism might be that the thiazides gave rise to alkalosis The latter would then neutralize the metabolic acidosis caused by the diabetes

This hypothesis does not appear very justifiable Firstly the thiazides produce a slight alkalosis only which can hardly neutralize a severe diabetic acidosis secondly a neutralization per se of the acidosis would not be able to remove the ketones from the blood and therefore could not prevent the appearance of acetonuria

Another hypothesis advanced by Antoniades (1) is based on the fact that the disease occurs in patients with maturity onset diabetes Such patients are known to be able to produce insulin (4) Antoniades believes that in these patients the insulin in plasma is bound to protein which would reduce the influence of insulin on muscle tissue and consequently the uptake of glucose in such tissue On the other hand in the fat tissue a factor is assumed to be present which is able to split the insulin from the protein so that the influence of insulin in fat tissue is maintained This results in inhibition of lipolysis and therefore also of the formation of ketone bodies In this way this hypothesis could explain the occurrence of hyperglycaemia without ketosis However the hypothesis is purely speculative based on experiments in vitro on animal tissue From experiments in vivo it was not possible to demonstrate any insulin effect on fat tissue of plasma from a patient with d c w k (14)

Up to the present the hypothesis advocated by Benedetto et al (2) is the most plausible These authors collected 27 cases of d c w k. from the literature and demonstrated firstly that the disorder is most common among elderly obese patients and secondly that prior to the development of coma many of the patients had consumed large quantities of carbohydrates Since in such patients administration of carbohydrates will cause an increase in the serum insulin (4 19) the initial content of insulin in the tissues will be high and this prevents the development of ketoacidosis Furthermore the insulin reserve in these patients is reduced (19) and consequently continued ad

ministration of carbohydrates must be supposed to exhaust the beta cells so that the production of insulin is reduced without necessarily being stop ped entirely This intensifies the hyperglycaemia Hyperglycaemia per se inhibits the formation of ketone bodies (16) The development of keto acidosis during this stage is thus counteracted by the small amount of insulin present in combina tion with the pronounced hyperglycaemia

From this hypothesis it will be po sible also to explain why d c w k is a frequent occurrence in patients treated with thiazides As the result of experiments both in vitro (9) and in vivo (21) it has been shown that thiazides inhibit the secretion of insulin from the beta cells Therefore it is reasonable to suppose that in diabetics treated with thiazides even relatively small amounts of carbohydrates are able to exhaust the beta cells and thereby to give rise to d c w k

The mechanism described may explain the de velopment of d c w k in all the patients com prised in our studies In two of the patients dia betes mellitus had been diagnosed previously One of these patients disregarded the dietary regula tions completely while the other had received a thiazide compound Diabetes mellitus had not been diagnosed previously in the remaining three patients Therefore they had not been on diet and in addition had been treated with thiazides

Treatment must be directed against the hyper glycemia and the dehydration To achieve this intensive fluid therapy and often high doses of insulin are needed (27) Whereas the insulin treat ment has not given rise to any major problems opinions differ as to which fluids should be used for infusion Sament (22) and Haapanen (11) think that isotonic sodium chloride solution should be employed also in cases when the serum sodium is increased Their reason is that apart from the fluid deficiency there is also a genuine lack of salt which is only masked by the pronounced de hydration

Other authors (12 13) are opposed to treat ment with isotonic sodium lactate sodium chlo ride and sodium bicarbonate as they claim that these fluids will cause an increase in a possibly existing hypernatremia and be unable to correct the hyperosmolarity Hence Halmos et al (12) suppose that the fatal outcome in two of their four cases might have been avoided if instead of saline and sodium lactate the patients had been given isotonic glucose which in their opinion is the correct fluid to be used for infusion Nelson (17) elaborates further on this assumption em phasizing that first and foremost these patients require water When given insulin intravenously together with the glucose solution the glucose will be assimilated almost as quickly as it is in fused and water will become available

Because patients with d c w k suffer from pro nounced water deficiency and since possibly there is a genuine but relatively smaller salt de ficiency it appears most rational to treat with hypotonic electrolyte solutions as recommended by e g Christiansen et al (5) and Bradley (3) Sjoberg (25) who also used hypotonic saline suspected that this solution gave rise to haemo lysis in one of his three patients but he does not state the quantities and concentrations of the fluid or the infusion rate

Initially our patients were treated in the same way as our comatose patients with ketoacidosis The three most seriously ill patients were given a hypotonic solution (composition (mEq/l) Na^+ 100 Cl^- 70 lactate$^-$ 30) and the other two were treated with an isotonic solution which was hypo osmolar in respect to electrolytes (composition (mEq/l) Na^+ 45 K^+ 25 Mg^{++} 5 Cl^- 45 $H PO_4^-$ 10 acetate$^-$ 20 sorbitol 50) The content of lac tate and acetate respectively in these infusion solutions is justified in patients with ketoacidosis but not in patients without ketoacidosis In pa tients without ketoacidosis we consider it more appropriate to use a hypotonic sodium chloride solution e g containing 100 mEq Na^+ and 100 mEq Cl^- per litre In other diseases we have ex ten ively used solutions in similar concentrations without encountering complications therefore the risk of haemolysis cannot be great

Even if the mortality observed in our material is considerably lower than average with only one death among the five patients no conclusions can be drawn as to the efficiency of the treatment on the basis of the results obtained firstly be cause the material is very limited and secondly because all the cases were mild

The personal experience gained by other authors is also very limited and their principles of treatment are based more on theoretical con siderations than on experience

Therefore it is important that this condition is recognized the diagnosis established early proper

treatment instituted and the result published in order that an efficient and well founded therapy may be developed and the high death rate reduced

REFERENCES

1 Antoniades H N Lancet 2 60? 1967
2 Benedetto R J Croco J A & Soscia J L Arch intern Med 116 74 1965
3 Bradley R F Med Clin N Amer 49 961 1965
4 Buchanan, K. D & McKiddie M T Diabetes 16 466 1967
5 Christiansen I Dalgård O Z. & Kjerulf K Nord Med 73 504 1965
6 De Graef J & Lips J B Acta med scand 157 71 1957
7 Dreschfeld J Brit med J 2 358 1886
8 Frerichs F T Z klin Med 6 1 1883
9 Frerichs H Reich U & Creutzfeldt W Klin Wschr 43 136 1965
10 Grønbæk, P & Kjeldsen F Ugeskr Læg 127 830 1965
11 Haapanen E Lancet 1 1154 1966
1 Halmos P B Nelson J K & Lowry R. C Lancet 1 675 1966
13 Hansen, E Ugeskr Læg 3 1097 1964
14 Jackson W P U & Forman R. Diabetes 15 714 1966
15 Joslin E P The treatment of diabetes mellitus 2nd ed p 88 Lea & Febiger Philadelphia and New York 1917
16 Mirsky I A Heiman, J D & Brok Kahn R Amer J Physiol 118 ?90 1937
17 Nelson J K. Lancet 1 1376 1966
18 Olafsson O & Sigfusson N Nord Med 77 496 1967
19 Perley M & Kipnis D M Diabetes 15 867 1966
?0 Rosenbloom, J N Y med J 102 294 1915
21 Samaan N Dollery C T & Fraser R Lancet 2 1 44 1963
22 Sament S Lancet 1 1153 1966
23 Sament S & Schwartz, M B S Afr med J 31 893 1957
24 Schwab R H Perloff J K & Porus R L Arch intern Med 111 465 1963
25 Sjoberg K H Lakartidningen 63 2913 1966
?6 Warburg E Acta med scand 61 301 19?5
27 With T K Nord Med 68 971 196?

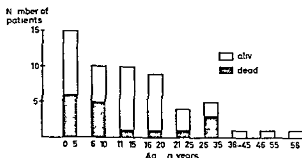


Fig 1 Total material Age distribution related to mortality

tion Out of the 56 cases included in the following presentation, 19 were females and 37 males The age at operation is shown in Fig 1 Three adult patients had a history of subacute bacterial endocarditis before the total correction

PRE-OPERATIVE FINDINGS

Twenty one patients were grouped in function class II nineteen in class III and sixteen patients in class IV No patient belonged to function class I

The localization of the systolic murmur was determined and its intensity graded according to Levine and Harvey (17) The maximum intensity was as a rule noted at the left sternal border in the third to fifth intercostal space Table I shows the character of the murmur in 55 of the 56 patients In the youngest patient fifteen months at operation no murmur could be heard The systolic murmur was of grade 3 or 4 in 46 cases and pansystolic in 31

The hematocrit reading was 45 or less in six patients 46 to 55 % in 22 and between 76 and 85 % in six

Table I *The systolic murmur in 55 cases of Fallot s anomaly prior to total correction*

Grade	Early systolic	Midsystolic	Late systolic	Spindle shaped	Pansystolic max: Early systolic	Midsystolic	Late systolic	Total
II	4	0	0	1	0	0	0	5
III	7	2	0	5	6	5	2	27
IV	1	1	0	2	3	9	2	18
V	0	0	0	0	1	2	1	4
VI	0	0	0	0	1	0	0	1
Total	12	3	0	8	11	16	5	55

Table II *The systolic pressure gradient RV PA (mm Hg) in 36 cases of Fallot s anomaly*

Systolic pressure gradient	No of pat	Systolic pressure gradient	No of pat
RV PA			
60–70	8	111–120	5
71–80	8	121–130	1
81–90	9	131–140	1
91–100	0	141–150	2
101–110	2	Total	36

Thrombocytes less than 100 000/mm³ were noted in six cases Three of these had a thrombotest count less than 40 In two cases the serum bilirubin was elevated and two had pathological bromsulphalein tolerance tests In all six cases a severe cyanosis was present and the hematocrit reading was 70 % or more

Definite electrocardiographic signs of right ventricular hypertrophy occurred in 41 cases and were suspected in 14 other cases Hypertrophy of the right atrium was electrocardiographically evident in 21 cases

The relative heart volume was normal or slightly elevated in most cases The four oldest patients had a more pronounced enlargement of the heart

At right heart catheterization it was possible to pass to the pulmonary artery in 36 out of 56 cases The peak systolic gradient between the pulmonary artery and the right ventricle is shown in Table II In 25 cases the gradient was between 60 and 90 mm Hg and in 11 cases more than 100 mm Hg

With the aid of data from the heart catheterization the angiocardiography and the findings at operation the pulmonary stenosis could be evaluated One patient had an isolated valvular stenosis 21 patients isolated infundibular stenosis In 34 cases the combination of valvular and infundibular stenosis was present

Fifteen patients had supravalvular malformations of the pulmonary artery as well In eight cases hypoplasia of the main stem was present and three of these had hypoplasia or aplasia of the left branch as well Two cases had hypoplasia of the left branch and three cases malformation of only the right branch In two patients finally

Table III *Vascular congenital malformations in 56 cases of Fallot s anomaly*

Atrial septal defect	2
Fistula from left coronary artery to bronchial artery	3
Duplication of right coronary artery	1
Aplasia of the pulmonary valves	2
Bicuspidal pulmonary valves	1
Bicuspidal aortic valves	1
Anomalous pulmonary vein to superior vena cava	1
Left sided superior vena cava	5
Situs inversus	1

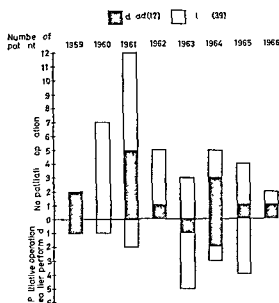


Fig Fifty six cases of Fallot s anomaly Total correc tion performed in 1959–1966

both pulmonary branches were malformed but not the main stem

Table III shows other malformations of the cardiovascular system Out of the two patients with atrial septal defect one had bicuspid aortic valves and one had persistent left superior vena cava

PER OPERATIVE AND IMMEDIATE POST OPERATIVE FINDINGS

Fig 2 presents the number of patients operated on each year and the mortality year by year Seventeen patients died after operation three of them immediately after the total correction and the other 14 within three and a half months after the operation Of the 17 cases ten were women and seven men

In the sequel the factors which might be of importance for the operative results will be com mented on These factors are chiefly the age at total correction the severity of the pulmonary stenosis the type of complicating cardiovascular anomalies the preceding palliative operation and finally the technique at operation including the length of the extra corporeal circulation and the primary result with regard to the pressure in the right ventricle

The median age of the 17 patients dying after operation was 7 years (range 1–34 years) com pared to the medium age of 11 5 (range 1–56 years) of the whole material The median age for the surviving cases was 12 years (range 2–56 years) From Fig 1 it is clear that the age group of 10 years or less at operation represents one third of the material and two thirds of the mor tality

Palliative treatment had been performed in 16 cases (Fig 2) Twelve of these were operated on by the Blalock Taussig procedure and in four cases the shunt was non functioning at the time of total correction In three cases the procedure of Brock had been used and in one case an aortico pulmonary window according to Potts had been made Of the 16 cases treated palliatively four died at the total correction The corresponding figures for the material not earlier operated on were 40 and 13 respectively

Fig 3 shows that when the functional capacity is low the mortality increases The hematocrit value could be taken as a sign of the severity of the heart disease Thus it was found that in the group with the highest hematocrit readings six patients with values between 76 and 85 four patients died and in the group of six patients having hematocrit readings less than 45 only one died In the group of 22 patients with hema tocrits between 46 and 55 seven died at or after the total correction

During the period 1959–1961 25 patients were operated on Ten of these cases (40°) had total AV block at the correction in five cases the block was not permanent During the period 1962–1966 31 patients were operated on and complete AV block occurred in four cases (13°₀) In the group of surviving patients for the whole period 1959–1966 total block occurred in seven (18)

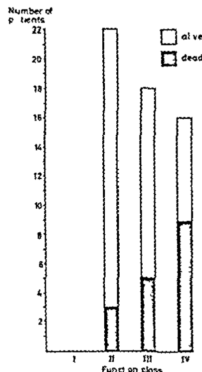


Fig 3 Mortality related to function class in 56 cases totally corrected for Fallot s anomaly

and in the group dying at or after operation complete AV block occurred in seven (41 %)

At the registration of the pressure in the right ventricle immediately after the correction the pressure was found to be reduced to 50 % or more in 26 of the 39 surviving cases (70 %) as compared to 5 of 17 (30 %) in the group of patients dying at or after operation Two of the 11 patients with a systolic pressure gradient more than 100 mm Hg pre-operatively died at the total correction

The perfusion time during the extra-corporeal circulation was 82 6 min (49–124 min) in the group of survivals and 86 3 min (47–134 min) in those who died

The main cause of death was septicemia in two cases in two other cases brain damage probably caused by the deep hypothermia was judged to be the cause of death

Of the 34 patients with a combination of valvular and infundibular stenosis ten died Of the 21 patients with an isolated infundibular stenosis seven died Eight of 15 patients with supravalvular changes of the pulmonary artery died at the operation In the group of 15 patients with other vascular malformations eight died at operation Six patients had both supravalvular pulmonary malformations and other vascular anomalies four of these patients died

FOLLOW UP EXAMINATION

Thirty six of the surviving 39 patients were investigated post operatively after between 5 and 82 months (medium 31 months) In three cases it was not possible to perform a complete post operative examination

The hematocrit value was normalized in all cases except in the 43 year old man whose value still was 53 % The explanation of his high hematocrit value is probably that he has a cystic renal disease His nonprotein nitrogen value was 55 mg % No shunt could be demonstrated at post operative cardiac catheterization or cardioangiography The arterial oxygen saturation was over 90 %

In the six cases with decreased thrombocyte values and also signs of increased bleeding tendency the thrombocytes increased to normal during the immediate post operative period

One case had a complete block post operatively and an atrial triggered pacemaker was implanted with good result Before the implantation the heart volume was 800 ml/m of body surface and after implantation normal Another case was re operated because of aneurysm of the outflow tract the outflow prosthesis of pericardium was changed to a prosthesis of teflon instead At re operation a small residual ventricular septal defect was noted the defect was resutured and the post operative course was uneventful The oldest case a 56 year old man was found to have a residual left to right shunt of 3 l/min at rest The arterial oxygen saturation was 98 at rest and 96 % at work Angiocardiography from the left ventricle showed a 3–4 mm wide communication from the right sinus Valsalvae to the outflow tract of the right ventricle No ventricular septal defect could be demonstrated Having regard to the age of the patient and the relatively good post-operative condition no new operation has been performed on this patient At the autopsy of a 4 year-old boy dying three months after total correction a small ventricular septal defect was found This patient who had signs of extra pyramidal lesions post operatively was found to have a diffuse brain

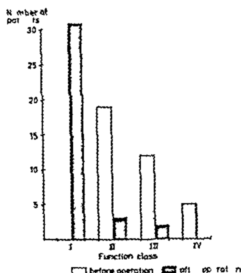


Fig. 4 Function class before and after total correction in 36 cases of Fallot's anomaly

lesion with destruction of the ganglionic cells especially in the pallidum as found in hypoxia

When grouping the patients in function classes 31 of 36 were found to be in class I post-operatively. Twenty patients changed from class II to I, nine from class III to I and two from class IV to I. The two cases having function class III post

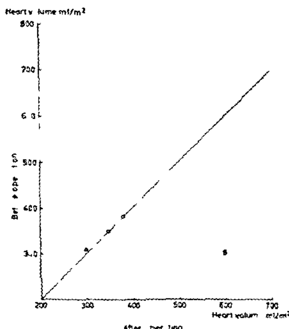


Fig. 5 Heart volume before and after total correction in 34 cases of Fallot's anomaly

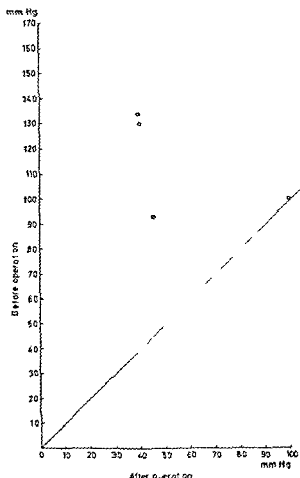


Fig. 6 The right ventricular systolic pressure at rest in 30 cases of Fallot's anomaly before and after total correction

operatively were the two oldest 55 and 56 years of age at the time of operation. None of the 36 cases had deteriorated (Fig. 4)

A complete right bundle branch block was noted in all cases except in a 22 year-old man who still had sinus rhythm post-operatively. In another patient bundle branch block was not present immediately after operation but was noted six months after operation and onwards.

The relative heart volume had increased moderately in 19 patients, was unchanged in ten and had moderately decreased in five (Fig. 5)

At the post-operative cardiac catheterization an overall good reduction of the right ventricular pressure at rest was noted (Fig. 6). A 3 year-old girl showed a pressure of about 100 mm Hg both

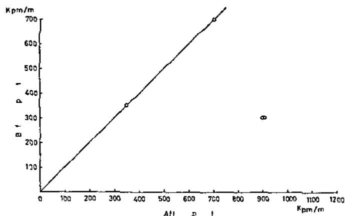


Fig 7 Working capacity in 18 cases of Fallots anomaly before and after total correction

pre and post operatively She was in function class II before and class I after operation

An improvement of the physical working capacity was obtained in most of the 18 patients whose working capacity could be compared before and after operation (Fig 7)

DISCUSSION

The mortality at total correction of Fallots anomaly varies in different materials Kirklin et al (14) had a mortality of 17 % in a group of 110 patients operated on during the period 1958–1959 If only the cyanotic cases were included the mortality in this material was found to be 23 % Bahnson et al (1) report a material of 147 cases with total correction performed between 1956 and 1961 In the cyanotic group the mortality was 32 % and in the non cyanotic 22 % In a Swedish material including 94 cases operated on from 1956 to 1962 Ekestrom (8) noted a mortality of 43 % Zerbini et al (25) had a mortality of 31 % during the period 1958–1961 but only 13 5 % for the period 1962–1964 Neutze (21) reported a mortality of 17 % in one hundred patients totally corrected 1960–1964 Barnard and Schrire (2) finally had a mortality of only 12 % in a material including one hundred patients corrected 1961–1965

The mortality of 30 % in our material is in rough accordance with earlier reports in which increased mortality was found in groups with severe disease and the highest mortality in the group with the most severe cyanosis The finding of the lowest mortality in the age group 11–25 years is in accordance with earlier reports This group which consisted of 23 patients in the present material had a mortality of only three cases

Two main causes of death can be seen surgical complications and factors connected with the nature of the material

The two cases of septicemia and the two cases of brain damage after deep hypothermia belong to the first group Deep hypothermia is not used any longer In one case in which a big coronary artery was severed the death was due to right ventricular failure In several other cases in which such an evident local cause could not be found right heart failure was probably a main factor contributing to the fatal outcome The type of incision in the heart is of interest not only from the point of view of surgical technique but also from the hemodynamic aspect There is no doubt that the longitudinal incision in the right ventricle originally used makes the operation technically easier From the aspect of the arrangement of the muscle fibers and the coronary vessels a transverse incision seems to be less harmful and has been said to involve less risk for right heart failure (10) In the present material a longitudinal incision was used in the first part of the series and later a transverse incision was performed or even an incision from the right atrium In this small material it is difficult to estimate the importance of the type of incision but a certain trend in favor of the transversal incision can be traced In the group of patients surviving a longitudinal incision had been made in three of five cases and a transversal or a right atrial incision in two of five The corresponding figure for the mortality group was four of five and one of five respectively

In the present series anoxic arrest with moderate hypothermia and also in some cases local hypothermia of the heart was used It would possibly have been preferable to use shorter periods of arrest not exceeding five minutes of the coronary artery flow combined with moderate hypothermia though this factor can hardly be of major importance An argument in favor of intermittent shorter periods of arrest is that the risk of surgical heart block will be reduced when operation is performed with beating heart allowing continuous control of the ECG The occurrence of complete AV block is always a latent risk at operation a risk that should be redu ed with increased technical experience which could also be noted in our series Even if it is possible to treat the block with pacemaker it is a serious complication which has probably contributed to the mortality In the group of surviving patients a complete block was found in seven cases (18) and in the group dying after operation it was also found in seven cases (41)

For obvious reasons the pressure measurements made at operation have limited significance A reduction of the right ventricular pressure to 50 or more of the systemic pressure was however twice as usual in the group of survivals as in the mortality group The perfusion time was not significantly different in the two groups Probably the duration of the p rfusion indicates the severity from a technical point of view and should per se not be of prognostic significance

The other main factor in the analysis of the mortality was the characteristics of the individual cases As mentioned above the greatest mortality was noted in the youngest patients among whom five of eleven operated on before the age of five years died In these cases it should thus be advisable to give a palliative treatment usually an anastomosis of the Blalock Taussig type as a first surgical procedure This has also been our policy during the last years Left heart failure was estimated to be a contributing cause of death in at least three cases It is possible that this could have been avoided by an earlier palliative treatment In the surviving group palliative treatment had been performed in one third of the cases and in the mortality group in one sixth Thus an earlier palliative treatment did not seem to involve an increased operative mortality

The greatest risk in primary total correction is then noted in the youngest children with severe stenosis in whom also an outflow prosthesis was necessary Wolf et al (24) among others have reported the greater mortality in cases with outflow prothesis The same tendency could possibly be traced in our material in which seven of the 22 cases with outflow prosthesis died

The three oldest patients 43 55 and 56 years old at operation all survived None of them had earlier been treated with palliative surgery Cooley et al (4) reported seven cases totally corrected of whom all were more than 35 years old Two of these died at operation and they were cyanotic Friesinger and Bahnson (9) report a successful total correction in a 54 year-old man Probably an increased cyanosis as a sign of the severity of the pulmonary stenosis will increase the operative risk in the higher age groups It is possible that the oldest patients with the anomaly of Fallot have less severe pulmonary stenosis in earlier years and the gradual increase of the stenosis could then be explained by an increase of the muscular hypertrophy of the outflow tract These cases are not cyanotic from the beginning but turn out to be cyanotic when the stenosis is so severe that the shunt becomes reversed A good collateral circulation from the bronchial arteries has also been ascribed as a prerequisite for lengthy survival in Fallot's anomaly

In eleven patients a systolic pressure gradient over the pulmonary valve of more than 100 mm Hg was noted two of these cases died at the operation As the number of cases in which the gradient measured pre-operatively is limited it is difficult to say whether a large gradient and high mortality are correlated In contrast to the finding of Wolf et al. (24) we could not find any definite increased mortality in patients with both valvular and infundibular stenosis It is also clear that the mortality increases if there are supravalvular changes of the pulmonary artery and also other vascular malformations

The patients surviving total correction show a definite improvement which could also be demonstrated objectively The hematocrit reading was normalized except in one case with a cystic congenital renal disease The low thrombocyte values in six patients became normalized after operation Somerville et al (22) investigated 50 cases with congenital heart disease with 40 cyanotic cases in the group In the cyanotic group

a tendency to thrombocytopenia and deficiency of fibrinogen was noted The most frequently encountered abnormality was depression of thromboplastin generation The degree of impairment of thromboplastin generation was greatest in the patients with the greatest increase in hemoglobin No abnormality was found in the ten patients with acyanotic congenital heart disease Kornhuber and Gutheil (16) are of the opinion that the oxygen consumption of the thrombocytes is high Hypoxemia as in cyanotic heart disease should therefore give a shorter survival time and in the most hypoxic cases a thrombocytopenia is also noted

The post-operative right heart catheterization showed a good reduction of the right ventricular pressure in most cases Yet most cases still had an increased right ventricular pressure which could be explained by the fact that part of the stenosis persisted

No patient was reoperated because of reappearance of ventricular septal defect The explanation is probably that most cases were operated on with a prosthesis instead of direct suture At the reoperation of the 7 year old boy for an aneurysmatic dilatation of the outflow prosthesis a minimal defect was found at the place where the ventricular septal defect had been sutured directly In this patient his pericardial outflow prosthesis was changed to a prosthesis of teflon From the experience of this and several other cases we believe that an outflow prosthesis from pericardium is less suitable

The relative heart volume increased moderately after operation in about half of the material Wolf et al (24) have earlier shown that cases with outflow prosthesis and pulmonary insufficiency more often get an increase of heart volume We could not find any definite correlation between heart size and outflow prosthesis in this material Another explanation of the increased heart volume could be reduced myocardial function by the incision in the right ventricle Miller et al (20) found left ventricular volumes at the lower normal limit in 12 of 14 cases of non-operated Fallot s disease It is reasonable to assume that the total correction gives an increased volume of the left ventricle secondary to the increased flow through the lungs

REFERENCES

1 Bahnson H T Spencer F C Landtman B Wolf M D Neill C A & Taussig H B Surgical treatment and follow up of 147 cases of tetralogy of Fallot treated by correction J thorac cardiovasc Surg 44 419 1962
2 Barnard C N & Schrire V The surgical approach to tetralogy of Fallot S Afr med J 40 330 1966
3 Bjork, V O & Hultquist G Brain damage in children after deep hypothermia for open heart surgery Thorax 15 284 1960
4 Cooley D A Hallman G L & Hammam A S Congenital cardiovascular anomalies in adult Results of surgical treatment in 167 patients over age 35 Amer J Cardiol 17 303 1966
5 Criteria committee of The New York Heart Association Diseases of the heart and blood vessels 6th ed., p 110 Little Brown & Company Boston 1964
6 Drew C E & Anderson I M Profound hypothermia in cardiac surgery Lancet 1 749 1959
7 DuShane J W & Weidman W H Five congenital cardiac defects A study of the profile and natural history Circulation Suppl 3 1965
8 Ekestrom S Results of open correction of the tetralogy of Fallot Acta chir scand 127 199 1964
9 Friesinger G C & Bahnson, H T Tetralogy of Fallot Report of case with total correction at 54 years of age Amer Heart J 71 107 1966
10 Gerbode F & Kerth W J Technical considerations in the treatment of tetralogy of Fallot the transverse ventriculotomy Ann Surg 158 975 1963
11 Harley H R S What is Fallot s tetralogy? Amer Heart J 6? 729 1961
12 Holmgren A & Mattsson K H A new ergometer with constant work load at varying pedalling rate Scand J Clin Lab Invest 6 137 1954
13 Jonsell S A method for determination of the heart size by teleroentgenography Acta radiol (Sto kh) 20 325 1939
14 Kirklin J W., Payne W S Theye R A & DuShane J W Factors affecting survival after open operation for tetralogy of Fallot Ann Surg 152 485 1960
15 Klinner W & Zenker R Experience with correction of Fallot s tetralogy in 178 cases Surgery 57 353 1965
16 Kornhuber B & Gutheil H Gerinnungsstorungen bei Kindern mit angeborenen Herzfehlern Z Kreisl Forsch 54 738 1965
17 Levine S A & Harvey W P Clinical auscultation of the heart 2nd ed Saunders Philadelphia 1959
18 Lillehei, C W Cohen M Warden H E & Varco R L The direct vision intracardiac correction of congenital anomalies by controlled cross circulation Results in 32 patients with ventricular septal defects tetralogy of Fallot and atrioventricularis communis defects Surgery 38 11 1955
19 Lillehei C W Morris J L Adams P & Anderson R C Corrective surgery for tetralogy of Fallot Long term follow up by postoperative recatheterization in 69 cases and certain surgical considerations J thorac cardiovasc Surg 48 556 1964

0 Miller G A H Kirklin J W Rahimtoola S H & Swan H C Volume of the left ventricle in tetralogy of Fallot Amer J Cardiol 16 488 1965

21 Neu ze J M Open heart surgery for tetralogy of Fallot N.Z med J Suppl 64 19 1965

? Somerville J McDonald L & Edgill M Post operative haemorrhage and related abnormalities of blood coagulat on in cyanotic congenital heart disease Brit Heart J 7 440 1965

3 Wahlund H Determination of the physical working capacity Acta med scand Suppl 15 1948

4 Wolf M D Landtman B Neill C A & Taussig, H B Total correction of tetralogy of Fallot Follow up study of 104 cases Circulation 31 385 1965

25 Zerbini E J Macruz, R., Bittencourt D Jaten A & Moura Campos Filho C Total correction of complex of Fallot under extracorporeal circulation Immediate results in a group of 2?1 patients J thorac cardiovasc Surg 49 430 1965

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ORGAN SPECIFIC INHIBITION OF THE IN VITRO MIGRATION OF LEUCOCYTES IN HUMAN GLOMERULONEPHRITIS

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Abstract The in vitro reactivity of peripheral leucocytes to renal parenchymatous homogenate is examined in 18 normal persons and 34 patients with nephropathia by means of a capillary tube migration technique In patients with active glomerulonephritis the migration is inhibited if the culture medium contains a homogenate of fetal kidney A similar inhibition is not seen with leucocytes from normal persons or patients with terminal nephropathy or pyelonephritis In brucella hypersensitivity a specific antigen induced inhibition of the in vitro migration of peripheral human leucocytes has been shown in the same experimental system, to be a parameter of cellular hypersensitivity The reactivity demonstrated in the present study may thus indicate that there exists in active glomerulonephritis a state of organ specific hypersensitivity of the cellular type directed to antigenic compounds in normal renal parenchyma

It is generally accepted that some kind of specifically altered reactivity induced by streptococcal antigen is pathogenetically important in acute glomerulonephritis although the mechanism initiating glomerular damage is still insufficiently explained On the basis of present knowledge an antigen antibody reaction between foreign—possibly glomerulus associated—protein and a specifically reactive immunoglobulin synthetized by the organism may be assumed to cause the initial glomerular lesion (4 17 21 22)

A similar *humoral hypersensitivity* reaction to foreign antigen however does not satisfactorily explain the progressive renal involvement which may continue for months or years in chronic active states of glomerulonephritis to all appearance independent of the initially important bacteria To explain the pathogenetic factors behind this course theories of auto-immunity have been advanced and have found some experimental support (7 9 13 14 15 16 18 19 20 31) According to these humoral as well as cellular hypersensitivity mechanisms may be considered instrumental in the development of protracted renal damage

In patients with glomerulonephritis the presence of circulating antibodies with specific reactivity to renal parenchyme components indicates that auto immune mechanisms play some part in the pathogenesis and the deposit of antigen antibody complexes in the glomerular membrane and capillary tufts seems to be an important pathogenetic feature (8 9) This however does not exclude the possibility that a co-existing organ-specific hypersensitivity of the cellular type may be pathogenetically instrumental (1 15 31)

A state of *cellular hypersensitivity* directed towards antigenic components of renal parenchyme would be tantamount to the presence in the organism of immunocompetent cells possessing a specific capacity to seek out and react with antigen-containing renal structures Attempts to demonstrate this type of reactivity in patients with glomerulonephritis by examining the intracutaneous delayed type reaction to human renal extract have not been confirmative (30) and a more exact evaluation of organ-specific cellular reactivity has so far not been possible in human glomerulonephritis mainly because an in vitro method for detection of cellular hypersensitivity in man has not been available

On the basis of methods developed by George and Vaughan (11) and David et al (5 6) the author suggested a technique for evaluating the in vitro migration of peripheral human leucocytes With this technique it could be demonstrated that the in vitro migration of leucocytes from brucella positive persons is specifically inhibited by brucella bacteria (28) Several observations indicate that specific antigen induced inhibition of immunocompetent cell migration is an in

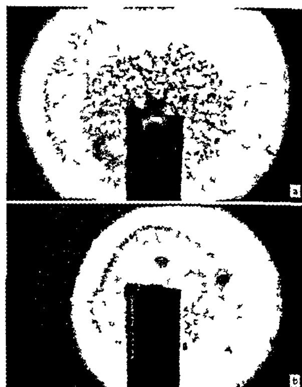


Fig 1 Leucocyte migration culture from case of active glomerulonephritis Migration period 24 hours (*a*) Control (*b*) Inhibition of migration induced by fetal renal homogenate

vitro correlate to cellular hypersensitivity (5 11 12 23 25 26 29) and this has been confirmed also with the in vitro technique employed in the present investigation (27 28) Further the present technique seems so far to permit an evaluation of cellular hypersensitivity in states which can not be estimated by more traditional methods e g auto immune diseases (2)

If there exists in glomerulonephritis a state of cellular hypersensitivity specifically directed to antigenic compounds in renal parenchyme one should expect renal parenchymatous components to induce an inhibition of the in vitro migration of leucocytes from patients with active glomerulonephritis An experiment of this kind might throw new light upon the pathogenesis of glomerulonephritis Leucocytes from patients with pyelonephritis or states of terminal nephropathia with fibrotic renal tissue might beforehand be expected to behave differently in the test system and the examination might thus find diagnostic applications or be informative concerning the activity of the disease at a given time besides giving information concerning pathogenetic relations

MATERIAL AND METHODS

Material

1 Eighteen consecutive patients without demonstrable renal affection

2 Fifteen consecutive patients with active glomerulonephritis Renal biopsies in 14 cases showed proliferative glomerular affection in two membranous glomerular affection in three and co-existing changes of both types in nine cases Clinically eight patients had a nephrotic syndrome with a 24 hour renal protein loss of more than 3 5 g

3 *Nineteen consecutive patients of whom seven had* terminal nephropathia with maximally contracted kidneys of unknown etiology (no histology) seven chronic glomerulonephritis in terminal phase (four bioptically verified) four chronic pyelonephritis and one acute pyelonephritis Creatinine-clearance was below 20 *ml/min* in 15 patients and within the normal range in three cases of pyelonephritis

All diagnoses were made on the basis of generally accepted anamnestic and objective criteria and were unknown to the laboratory performing the leucocyte migration test (LMT)

Antigens

Tissue homogenates were prepared from lyophilized sterile fetal kidney liver colonic mucosa and ileal mucosa All preparations were standardized by simple quantitative protein determination The concentration of homogenate in the test cultures was with kidney and liver homogenates 160 μg with colonic or jejunoileal mucosa homogenates 80 μg protein per ml culture medium

Technique

Heparinized blood from a cubital vein is allowed to settle for one hour at 37 C The plasma with white cells is aspirated and the cells (ca 60% mononuclear 40% polymorphonuclear leucocytes) are washed four times in Hank s BSS and placed in capillary tubes The cells migrate from the free end of the tube into a 1 ml tissue culture chamber containing TC 199 culture medium with 10 horse serum After 24 hours the migration is visible as a nearly circular sheet protruding from the tube opening (Fig 1 *a* and *b*) and measurable by planimetry in a projection microscope On the basis of the average 24 hour migration area in a series of control cultures without antigen Mo and the average 24 hour migration area in a series of cultures containing the antigen Mx the migration index

$$MI = \frac{Mx}{Mo}$$

is calculated MI thus indicates an inhibition of the cell migration if the values are lower and a stimulation if greater than unity

RESULTS

The results are presented in Fig 2 The white cell migration of 18 persons without demonstrable renal affection (left column) appears to be

rather unaffected by the presence of renal homogenate The normal range (mean ± 2 S D) of MI is 0 83–1 11 The findings in 15 cases of active glomerulonephritis are indicated in the middle column Subnormal values of MI are found in 13 cases and the observations show a distribution significantly different from the normal material ($p < 0\ 001$) Despite three subnormal levels the mean white cell migration of patients with terminal nephropathia or pyelonephritis (right column) does not show any significant difference from the normal material but is significantly different from the group with active glomerulonephritis ($p < 0\ 001$)

In all cases showing inhibition with renal homogenate MI appeared to be quite uninfluenced by colonic or ileal mucosa homogenates In two cases of acute glomerulonephritis the leucocyte migration was inhibited by hepatic as well as renal homogenate

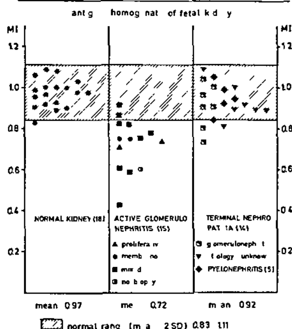


Fig 2 Leucocyte migration test (*LMT*) with homogenate of fetal kidney in 18 normal persons and consecutive cases of active glomerulonephritis (15) and terminal nephropathia or pyelonephritis (19) *MI* = migration index

DISCUSSION

In human glomerulonephritis several observations support the view that circulating antirenal immunoglobulin is an important cause of glomerular damage This does not exclude however that antirenal antibody may at the same time be regarded also as an epiphenomenon secondary to otherwise caused renal destruction Its very presence indicates that the organism contains immunocompetent cells specifically equipped to synthetize antirenal antibody It may consequently be supposed that other immunocompetent cells not necessarily engaged in antibody production but with the same specificity might possess the ability specifically to react with antigen-containing renal structures following the pattern of a cellular hypersensitivity reaction

Experimentally a combined proliferative and membranous glomerulonephritis can be induced in rats by injection of renal extract in Freund s adjuvant (14) In transfer experiments lymphocytes from rats with this kind of experimental glomerulonephritis seem to cause similar glomerular damage in normal recipient animals (13) Transferability by means of lymphocytes has been demonstrated in other kinds of experimentally induced auto-immune diseases (10 24) and is at the same time a fundamental feature of cellular hypersensitivity (3) Although these experiments are informative only of a special type of glomerulonephritis experimentally induced in rats they seem to indicate that a mechanism of organ specific cellular hypersensitivity can be instrumental in glomerular damage

From the present study it appears that the in vitro migration of leucocytes from patients with active states of glomerulonephritis is inhibited if the culture medium contains a homogenate of fetal kidney The migration of leucocytes from normal persons is uninfluenced by the homogenate and patients with pyelonephritis or terminal fibrotic nephropathia do not show any significant difference from the normal group The white cell reactivity to renal tissue components thus indicates a distinct difference between glomerulonephritis and the other renal diseases examined and discloses the presence in normal renal parenchyme of some compound which possesses the ability specifically to inhibit the migration of leucocytes from patients with glomerulonephritis

The specific antigen induced inhibition of leucocyte migration as studied by the present technique seems to be a reliable parameter of cellular

hypersensitivity (27 28) As to the specific reactivity to renal tissue components observed in the present experiments a similar view may be taken The results can accordingly be interpreted as indicating the existence in active human glomerulonephritis of a state of organ specific hypersensitivity of the cellular type directed to antigenic components of renal parenchyma Even if this type of autoreactivity might afford a good explanation of several pathogenetic relations in glomerulonephritis its importance as a pathogenetic factor cannot be evaluated on the basis of the present investigation

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REFERENCES

1 Bendixen G Classification of hypersensitivity in relation to clinical disease Ann intern. Med 64 658 1966
2. — Specific inhibition of the in vitro migration of leucocytes in ulcerative colitis and Crohn s disease Scand J Gastroenterol 2 214 1967
3 Chase M. W The cellular transfer of cutaneous hypersensitivity to tuberculin. Proc Soc exp Biol. (N Y) 59 134 1945
4 Cruickshank, B Nephritis nephrosis rheumatic fever and myocardial infarction. In Clinical aspects of immunology (eds. P G H Gell & R. R. A. Coombs) p 572 Blackwell Scientific Publications, Oxford 1962.
5 David J R., Al Askari S Lawrence H S & Thomas L. Delayed hypersensitivity in vitro I. The specificity of inhibition of cell migration by antigens J Immunol. 93 -64 1964
6 David, J R. Delayed hypersensitivity in vitro Its mediation by cell free substances formed by lymphoid cell antigen interaction Proc nat Acad. Sci (Wash.) 56 7-. 1966
7 Dinh, B L. & Brassard, A Induction of experimental glomerulonephritis in the rat by homologous major urinary protein. Clin exp Immunology 2 633 1967
8 Dixon, F J Autoimmunity Autoimmune glomerulonephritis 6th International Congress of Allergology Montreal 1967
9 Edgington, T S., Glassock, R. J & Dixon, F J Autologous immune-complex pathogenesis of experimental allergic glomerulonephritis Science 155 1432, 1967
10 Felix Davies D & Waksman, B H Passive transfer of experimental immune thyreoiditis in the guinea pig Arth. and Rheum. 4 416 1961
11 George M & Vaughan J H In vitro cell migration as a model for delayed hypersensitivity Proc Soc exp Biol (N Y) 111 514 1962
12 Heilmann, D H., Howard D H & Carpenter C M Tissue culture studies on bacterial allergy in experimental brucellosis I The effect of brucella suis whole antigen on cultures of spleen from normal and brucella infected guinea pigs J exp Med. 107 319 1958
13 Hess E V., Ashworth, C T & Ziff M Transfer of autoimmune nephrosis in the rat by means of lymph node cells J exp Med 115 421 1962.
14 Heymann, W., Hackel D B Harwood, S., Wilson, S G F & Hunter J L. P Production of nephrotic syndrome in rats by Freund s adjuvants and rat kidney suspensions Proc Soc exp Biol. (N Y) 100 660 1959
15 Holm, G In vitro cytotoxic effects of lymphoid cells from rats with experimental autoimmune nephrosis Clin exp Immunology 1 45 1966
16 Hunter J L P Hackel D B & Heymann W Nephrotic syndrome in rats produced by sensitization to rat kidney proteins Immunologic studies J Immunol 85 319 1960
17 Kaplan, M H Localization of streptococcal antigens in tissue I Histologic distribution and persistence of M protein, Types 1 5 12 and 19 in the tissues of the mouse J exp Med 107 341 1958
18 Lange K., Gold M M A Weiner D & Simon, V. Auto-antibodies in human glomerulonephritis J clin. Invest 28 50 1949
19 Lerner R. A. & Dixon F J Transfer of ovine experimental allergic glomerulonephritis (EAG) with serum. J exp Med 1-4 431 1966
20 Liu C T & McCrory W W Autoantibodies in human glomerulonephritis and nephrotic syndrome J Immunol 81 492 1958
21 Markowitz, A S Armstrong S H., Jr & Kushner D S Immunological relationships between the rat glomerulus and nephritogenic streptococci Nature (Lond) 187 1095 1960
22 Markowitz A S Clasen R Nidus B D & Ainis H Streptococcal related glomerulonephritis II Glomerulonephritis in rhesus monkeys immunologically induced both actively and passively with a soluble fraction from human glomeruli J Immunol 98 161 1967
23 Moen J K. Tissue culture studies on bacterial hypersensitivity III The resistance in vitro of the inherent sensitivity to tuberculin J exp Med. 64 943 1936
24 Patterson P Y Transfer of allergic encephalomyelitis in rats by means of lymph node cells J exp Med. 111 119 1960
25 Rich A R. & Lewis M R. The nature of allergy in tuberculosis as revealed by tissue culture studies Bull Johns Hopk. Hosp 50 115 1932
26 Švejcar J & Johanovský J Demonstration of delayed (tuberculin) type hypersensitivity in vitro I. Selection of methods Z. Immun Forsch 122 398 1961

27 Søborg, M In vitro detection of cellular hyper sensitivity in man Acta med scand 182 167 1967
28 Søborg, M & Bendixen, G Human lymphocyte migration as a parameter of hypersensitivity Acta med scand 181 247 1967
29 Thor D E Delayed hypersensitivity in man A correlate in vitro and transfer by an RNA extract Science 157 1567 1967
30 Wagner V & Rokop J Auto-antibodies and skin tests with extracts from normal kidney in cases of diffuse glomerulonephritis Int. Arch Allergy 9 231 1956
31 Waksman B H Tissue damage in the "delayed (cellular) type of hypersensitivity In. Mechanism of cell and tissue damage produced by immune reactions (eds P Grabar & P Miescher) p 146 B Schwabe & Co Basel 1962

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SERUM MONOAMINE OXIDASE (MAO) IN DIABETES MELLITUS AND SOME OTHER INTERNAL DISEASES

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Abstract Serum monoamine oxidase (MAO) activity has been studied in normal adults and in about 500 persons with diabetes mellitus and some other internal diseases We have confirmed earlier reports that serum MAO is increased in patients with chronic congestive heart failure and that the enzyme level is correlated to the degree of incompensation Increased serum MAO concentration was also registered in persons with thyrotoxicoses and diabetes mellitus Serum MAO activity was found to be increased already at the first appearance of diabetes mellitus and was not influenced by the type of therapy A decreased serum MAO activity was found during treatment of different diseases with corticosteroids and ACTH as well as in some cases of neoplastic diseases

As shown by McEwen and Cohen (5) human blood serum contains a soluble monoamine oxidase that catalyzes the oxidative deamination of several monoamines—i e benzylamine phenyl ethylamine tyramine dopamine and tryptamine (3) This enzyme is in many respects similar to mitochondrial MAO which however also catalyzes the oxidation of other amines i e epinephrine norepinephrine and serotonin It might be possible that serum MAO is an isoenzyme of mitochondrial MAO

It has been suggested that the serum MAO activity is independent of age sex and meals Unlike serum diamine oxidase serum monoamine oxidase is not elevated during pregnancy (2) In recent years it has been shown that serum MAO activity is increased in chronic congestive heart failure of different origin (6) in liver cirrhosis (4) and in diabetes mellitus (8) In addition the enzyme level has been reported to be increased in a few cases of thyrotoxicosis and carcinoid syndrome (6) Treatment with nitroglycerin decreases mitochondrial MAO activity (9) Consequently it might possibly also cause a decrease of serum MAO

We started this investigation with the general intention to study the variations of serum MAO activity in different clinical conditions We also intended to find out the value of serum MAO determination as a screening test for chronic congestive heart failure

MATERIAL

The material consisted originally of all 60 patients hospitalized on one occasion in a male and a female ward of the Department of Internal Medic ne After the find ng of divergent serum MAO alu s in diabetes mellitus myeloproliferative dis as s and thyrotoxicosis as well as under steroid treatment these special groups of patients have been further completed With regard to d abetes the large series of the hospital described by Nilsson et al (7) could be used The total number of persons investigated is about 500 For comparison with the f gures of McEwen the enzyme level has also been determined in 38 healthy blood donors

METHODS

Serum MAO activity was determined according to McEwen and Cohen (5) with benzylamine as the substrate Assay and control reaction were incubated in open test tubes at 37 C for 3 hours The assay tube contained 600 μl of serum 700 μl of 0 1 *M* phosphate buffer (pH 7) and 00 μl of 6 m*M* benzylamine hydrochloride (in the same phosphate buffer) At the end of the incubation time 00 μl of 50 per cent perchloric acid was added to stop the reaction In the control tube the substrate was added just before the perchloric acid 1 5 ml of cyclohexane was added to each tube and the contents of the tubes were emulsif ed with a super mixer" The tubes were allowed to stand for 15 minutes at room temperatu e and were then after a second emulsif cation, centrifuged in a cl n cal centrifuge After that the absorban y of the cyclohexane extract was measured at 4 nm w th a Zeiss PMQII spectrophotometer (10 mm cuvette) An enzyme unit was defined by McEwen as the difference in optical density between

Table I *Serum MAO in normal series and in various clinical states*

	n	Mean age (y)	MAO U (McEwen)	
			Range	Mean
A Blood donors	38	31 0	13 4–51 5	29 6
In patient not classed below	57	58 8	16 1–52 4	30 5
B Cardiac insufficiency	41	69 0	15 0–91 8	44 2
Degree of incompensation 1	23		15 0–63 6	37 9
2	7		34 3–57 8	43 5
3	8		21 2–91 8	56 5
4	3		57 0–68 2	62 5
Cardiac infarction	15	68 9	21 2–63 6	40 4
First infarction	9	70 8	21 2–49 4	33 1
Nitroglycerin treated	5			46 4
C Arterial hypertension	13	52 5	16 7–49 7	35 4
Treated with α methyldopa	7		32 0–44 9	34 3
D Thyrotoxicosis	8	62 5	39 4–87 7	57 8
E Corticosteroid treated	23	56 8	15 5–47 2	26 1
Without cardiac disease	17		15 5–34 5	22 2
Bronchial asthma	14			25 1
Rheumatic disease	2			16 3
Neurologic disease	1			18 4
F Leucemia malignant lymphoma and myeloma	8	63 6	10 7–42 0	21 2

assay and control multiplied by 100 According to the recommendations of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes a unit (U) is the amount of enzyme that catalyzes the degradation of 1 μmol of the substrate per minute under well defined conditions Consequently the MAO concentration can be expressed in μU/ml by multiplying the difference in optical density between assay and control by 1200 The figure has been found by determination of the extraction efficiency (about 80 per cent) of benzaldehyde in the procedure and the molar coefficient of extinction 1 4 × 10^4 *M* cm (at 242 nm in cyclohexane) The precision of the method in our hands is the same as reported by McEwen and Cohen (5)

The degree of cardiac incompensation has been described by the weight loss of the patient during the stay at the hospital Degree 1 has been defined as a body weight loss of less than 3 kg degree 2 between 3 and 7 kg degree 3 between 7 and 15 kg and degree 4 more than 15 kg

RESULTS AND DISCUSSION

The results of the serum MAO determinations have been summarized in Table I and with regard to diabetes mellitus in Table II

A *Normal values*

Our serum MAO values are in very good agreement with those reported by McEwen and Cohen (5) for the age 18 to 50 years The values found for healthy blood donors are not different from those found for in patients of the Department of Internal Diseases or the Department of Surgery without a clinical picture or treatment known to influence the serum MAO activity A significant increase of serum MAO was however found for children (1)

B *Chronic cardiac heart failure*

The degree of cardiac incompensation has been roughly estimated by determination of the body weight reduction during therapy In this way the correlation between the degree of heart incompensation and the serum MAO activity has been convincingly proved This is a confirmation of earlier results of McEwen and Harrison (6) who used exact hemodynamic measurements for the determination of the cardiac state When the patients with chronic congestive heart failure were dehydrated there was a tendency to lowering of the serum MAO values However they were not completely normalized An attempt was also made to correlate the serum MAO activity and the heart size determined by roentgenological techniques but this correlation was found to be very uncertain A group of patients with heart infarction was found to have almost normal serum MAO values at the time of the first heart attack In connection with reinfarction the degree of incompensation as well as the serum MAO level

Table II *Serum MAO in diabetes mellitus*

	n	MAO U (McEwen)	
		Range	Mean
H Diabetes mellitus	340	20 7–99 4	48 0
Males total	152	21 6–76 4	45 0
Age y 20–39	35	30 6–67 9	45 1
40–59	54	21 7–80 0	44 7
60–79	63	21 6–76 4	45 1
Females total	188	20 7–99 4	50 6
Age y 20–39	27	29 0–61 3	42 3
40–49	19	29 2–86 0	50 3
50–59	29	26 8–99 4	55 2
60–69	67	25 0–88 7	51 0
70–79	46	20 7–84 8	51 9
Insulin therapy	191	21 6–99 4	49 4
Insulin > 60 i u	26	23 0–67 9	50 5
Peroral therapy	149	.0 7–84 8	46 9
Newly discovered disease	24	19 2–92 0	48 5
> 15 years duration	34	23 0–73 0	50 6

were found to be elevated Therapy with nitro glycerin did not generally decrease the serum MAO activity However a few unexpectedly low enzyme values were registered for patients with such treatment The highest values found for the cardiac patients were 92 0 and 88 7 U (McEwen) These two patients were also diabetics Two other cardiac patients with exceptionally high values (87 7 and 73 4) were also suffering from thyrotoxicosis

C *Arterial hypertension*

In patients with arterial hypertension without known effects on the heart a very slight serum MAO increase was noted α methyldopa (0 25 g × 2–4) was not found to influence the enzyme level

D *Thyrotoxicosis*

As earlier reported by McEwen and Harrison (6) we found increased serum MAO values in con nection with thyrotoxicosis The material is yet too small for the determination of a possible correlation between the degree of thyrotoxicosis and the serum MAO level The lowest enzyme value in this group was registered for a patient with intensive symptoms of thyrotoxicosis factitia In two cases with extreme myxedema normal serum MAO activity was registered

E *Steroid treatment*

In connection with steroid treatment low serum MAO values have been registered with few ex ceptions Prednisolone has been given in doses of 2 5–5 mg × 1–3 for periods up to 3 years Treatment with ACTH has been given as oc casional therapy in status asthmaticus In three cases low values about 20 U (McEwen) were found already 2–3 days after treatment with ACTH (dose = 60 I U × 3) had started In a small subgroup of steroid treated patients with electrocardiographic signs of heart disease the MAO values were not especially low

F *Malignant diseases*

In a few cases of leucemia myeloma and malig nant lymphoma the serum MAO concentration was decreased to excessively low values

G *Nephrosis and myasthenia gravis*

Apart from the cases included in Table I there are two cases of special interest

Case 1 A woman 33 years old with nephrosis which appeared two months after a throat infec tion During treatment with ACTH the protein uria was reduced from 0 04 g/100 ml to 0 01 g/100 ml Serum MAO activity decreased from 106 2 U (McEwen) which is the highest value yet registered by us to 88 5 U (McEwen) after 10 days treatment and to 77 2 U (McEwen) after 20 days However it must be mentioned that another patient with a similar clinical picture of nephrosis had normal serum MAO values

Case 2 A woman 37 years old with a newly diagnosed myasthenia gravis initially had a serum MAO value of 54 6 U (McEwen) The enzyme level was unchanged after two months during which period the patient was successfully treated with mestionin Another case of myasthenia gravis a woman 38 years old had a serum MAO value of 39 5

H *Diabetes mellitus*

In diabetes mellitus there is a statistically significant increase of serum MAO concentration (8) Only 14 of the 340 persons in the diabetic group had serum MAO values below 30 U (McEwen) which was the normal mean value The diagnosis of diabetes mellitus was dubious in eight of these 14 patients because it was founded only on single pathological glucose tolerance tests or on solitarily increased blood sugar values Only two of the 95 control persons (group A) in this study had a serum MAO activity higher than 48 U (McEwen) which was the mean for the diabetics The increase seems to be independent of the duration of the disease In six patients with diabetes mellitus the increased enzyme value was registered already when the disease was first detected and diagnosed The serum MAO activity seems independent of the type of treatment and the dose of insulin and there seems to be no correlation to the development of complications Occasional insulin treatment for example in psychiatric diseases was not found to influence the serum MAO level Diabetics with chronic congestive heart failure or thyrotoxicoses were found to have especially high enzyme values Further studies on the correlation between serum MAO and glucose metabolism are in progress with special regard to diabetic gene carriers

REFERENCES

1 Berg R Nilsson S E Tryding, N & Tufvesson G To be published
2 McEwen C M Serum amine oxidases in pregnancy J Lab clin Med 64 540 1964
3 — Human plasma monoamine oxidase J biol Chem 240 2003 1965
4 McEwen C M & Castell D O Abnormalities of serum monoamine oxidase in chronic liver disease J Lab clin Med 70 35 1967
5 McEwen C M & Cohen J D An amine oxidase in normal human serum J Lab clin Med 62 766 1963
6 McEwen C M & Harrison D C Abnormalities of serum monoamine oxidase in chronic congestive heart failure J Lab clin Med 65 546 1965
7 Nilsson S E Nilsson J E Frostberg N & Emilsson T The Kristianstad Survey II Acta med scand Suppl 469 1967
8 Nilsson S E Tryding N & Tufvesson G MAO and diabetes mellitus Proceedings of the Sixth Congress of Internal Diabetes Federation Stockholm July 30 – August 4 1967 In print
9 Ogawa K Gudbjarnason S & Bing R. J Nitroglycerin (glyceryl trinitrate) as a monoamine oxidase inhibitor J Pharmacol exp Ther 155 449 1967

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LACTIC DEHYDROGENASE IN KIDNEY TISSUE AND RENAL DISEASE

Adaptive Change of the Synthesis in Acute Renal Failure

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Abstract The concentration of lactic dehydrogenase (LDH) and LDH isoenzymes was determined in different zones of normal human kidneys and in sera from patients with renal diseases

The isoenzyme pattern in renal tissue was characterized by an increasing relative concentration of the M subunit from the cortex to the papilla The outer non glomerular cortex corticis zone presented a medulla like isoenzyme pattern which has not been described hitherto This correlated well with new data on renal blood flow in this zone

Patients with chronic renal failure had normal or slightly elevated serum concentrations of LDH and the isoenzyme pattern was inconclusive

In patients with renal traumata, serum LDH concentration was very high but the isoenzyme pattern did not differ from the normal

In acute *oliguric* glomerulonephritis serum LDH was moderately elevated while LDH concentrations were usually very high in acute renal failure of widely different pathogenesis In both groups however the isoenzyme pattern exhibited a uniform relative increase of the M-subunit concentration Taking into consideration new data on (*a*) renal blood flow in acute renal failure and (*b*) adaptation of LDH synthesis to varying oxygen tension the hypothesis is advanced that the isoenzyme pattern reflects an altered synthesis of LDH in the kidneys as an adaptive response to hypoxia in the initial phase of acute renal failure

This report deals with studies of lactic dehydrogenase (LDH) and LDH isoenzymes in different zones of normal human kidney tissue and in serum from patients with acute and chronic renal diseases

In a previous paper (14) we reported on a considerable increase of LDH in serum from patients with acute renal failure We also demonstrated a uniform increase of all five isoenzymes consistently with the pattern obtained from whole kidney tissue homogenate

LDH is an intracellular enzyme in the energy metabolism and consequently it is found in almost all human tissues The concentration of LDH (units per gram tissue) however varies considerably in different tissues Apart from the retina the highest concentration is found in the kidney (40)

In normal serum the concentration of LDH and the isoenzyme pattern is supposed to be the result of a release of enzymes from different tissues Pathological states in tissues with a low concentration of LDH resulting in an increase of the serum *concentration* indicate a rather extensive cell lesion (i e necrosis or altered membrane permeability) as a liberation of 10 000 LDH units is required to cause an elevation of the concentration of *1* unit per ml in an extracellular volume of approximately *10* litres In tissues with a high concentration of LDH a far less extensive cell lesion would give the same result A change in the isoenzyme *pattern* in serum further requires that the isoenzyme pattern of the enzyme liberating organ differs considerably from that of serum in which case even minor contributions may cause a demonstrable change in the pattern of serum thus making the isoenzyme determination a more sensitive test than the total LDH determination (e g the protracted elevation of LDH_1 after myocardial infarction or of LDH_5 in subclinical or protracted hepatitis despite normal total activity (39)

During recent years the theory (1) of two types of LDH (H_4 and M_4) tetramerically constituted from two different monomers or subunits (H and M) has received great support from divergent physico-chemical properties (11 37) By hybridization of the two subunits into tetrameric molecules another three intermediate fractions

are formed (H_3M $H M_2$ and HM_3) (23) thus resulting in five electrophoretically separate proteins or isoenzymes The biologically most fundamental distinction between the two subunits appears to be the influence of the oxygen tension (pO) on the rate of synthesis of the subunits Experiments have shown that a low pO_2 in homogenous cell culture promotes the synthesis of the M subunit while a high pO_2 results in a preferential synthesis of the H subunit (4 6 8) On the basis of these data the Kaplan group advanced the *oxygen tension theory* according to which the rate of synthesis of the single subunits in cells is determined by the local pO This corresponds well with the observations that H_4 LDH works optimally under aerobic conditions with a low concentration of lactate while M_4 LDH is inhibited only by very high concentrations of lactate thus preserving its catalytic activity also under anaerobic glycolysis (11)

In this report the hypothesis is advanced that the synthesis of LDH in kidney tissue is altered as an adaptive response to hypoxia during the initial phase of acute renal failure (syn ischaemic nephropathy acute tubular necrosis ')

METHODS

The serum concentrations of LDH and glutamic pyruvic transaminase (GPT) were determined by fluorometric methods (18 19) The enzyme concentration was expressed as units per ml One unit was defined as the amount of enzyme which was capable of causing a reaction velocity of 1 μmol per hour Normal values were LDH = 7–21 units per ml (168 persons) and GPT = 0–1 8 units per ml (172 persons)

The determination of LDH isoenzymes was performed according to a technique originally described by Wieme (38) and later modified by Jensen and Laursen (10) Agarose was used as a supporting medium and NADH and pyruvate were used as substrate The relative distribution of the five isoenzymes was calculated by weighing the single fractions of the curve obtained by photometric scanning of the electrophoresis plate The total concentration of the H and M subunit as defined by Kaplan and Goodfriend (11) was calculated from the percentage distribution of the five isoenzymes The percentage of the total M subunit will be referred to as the MSU value The standard deviation on the difference between double determinations of the MSU value in 20 pathological sera was 2 8 %

The reliability of this method was checked by another more laborious quantitative method based on an extraction of the single isoenzymes followed by a quantitative measurement of the concentration in test tubes (20) In this method a standard deviation of 0 9 % was obtained on differences between 13 double determinations In nine sera both methods were applied The standard deviation on differences between the MSU values (ranging from 20 to 65 %) was 3 9 % This difference was insignificant ($P > 0\ 05$)

Sera from 30 healthy males between the ages of 20 and 30 years served as normal material Results appear from Table II

Kidney tissue preparation

Samples of human kidney tissue were cut into pieces and carefully rinsed in 0 9 per cent saline After homogenisation (400 rpm in a Potter Elverhjelm homogenisator for 2 min at 0 C) and centrifuging (at $2500 \times g$ for 10 min) the LDH isoenzymes in the supernatant were determined according to the above mentioned technique

MATERIAL

Twenty one samples of human *kidney tissue* were obtained from seven kidneys at autopsy (12–15 hours post mortem) There was no evidence of renal disease prior to death and the autop y did not reveal any pathological features in the examined kidneys

Three samples were cut tangentially in thin slices (1–2 mm) from the cortex corticis zone after removal of the fibrous capsule Nine samples from the cortical and six samples from the medullary (= non glomerular) zones were cut in varying depth Finally three samples were cut from the apex of the papilla after removal of connective tissue

Sera were examined in 74 patients admitted to our renal unit with different renal diseases The material was divided into four groups

A Acute renal failure 44 patients

B Renal traumata 8 patients

C Acute oliguric glomerulonephritis 4 patients

D Chronic renal failure 18 patients

In groups A B and C several determinations of LDH were made within the first week after

Table I *The concentration of lactic dehydrogenase and the relative distribution of isoenzymes in different zones of the kidney*

Kidney zone	*n*	Total LDH (unit/ml)	LDH isoenzymes (%) I	II	III	IV	V	M-subunit ( )	*P*
Cortex corticis	3	265 (228–340)[a]	30	30	21	13	6	34±1 4[b]	
									<0 005
Cortex	9	254 (60–3 8)	38	32	17	8	5	27±4 7	
									<0 001
Medulla	6	292 (112–387)	27	27	20	16	10	39±4 8	
									<0 005
Papilla	3	204 (82–293)	13	19	22	24	22	55±3 5	

[a] Mean value and range in brackets
[b] Mean value ± one standard deviation.

admission and the MSU values were calculated from the maximal LDH concentration recorded This was in every case the first value obtained

A *Acute renal failure* All patients fulfilled the diagnostic criteria of acute renal failure cited in our first report (14) The patients were subdivided into six groups as follows

I *Posttraumatic* (7 patients) Acute renal failure following traffic accidents Various and often widespread extrarenal lesions were present in all patients

II *Gastro intestinal tract surgery* (8 patients) Precipitating causes were Perforating acute appendicitis (four) perforation of gastroduodenal ulcer (three) and removal of rectal cancer (one)

III *Biliary tract disease* (7 patients) Anuria followed cholecystectomy

IV *Other surgical causes* (7 patients) Anuria followed other types of major surgery (vascular and lower urogenital tract surgery)

V *Infections* (9 patients) This heterogenous group comprised patients in whom severe infections were the dominant or the only precipitating cause

VI *Other medical causes* (6 patients) Primary disorders were Barbiturate intoxication haemolytic anaemia transfusion reaction dehydration diabetic coma and eclampsia

B *Renal traumata* Eight anuric patients are grouped under this heading as all of them presented necrosis of renal tissue in varying degree Only two of the patients had extrarenal lesions as well both following traffic accidents Their clinical course was indistinguishable from that of acute renal failure but autopsy revealed severe cortical infarction with widespread necrosis Another four patients showed uni or bilateral renal infarctions and necroses at autopsy as a result of renal artery occlusion Two patients survived one otherwise healthy patient was referred to us with post renal obstruction after accidental perforation of both kidneys (ureteral catheterisation) This patient was included in our first report (14) The other patient underwent nephrostomy in his single kidney because of post renal obstruction LDH was determined the day after this operation

C *Acute glomerulonephritis* Four patients developed oliguria in the initial phase of acute glomerulonephritis Glomerular filtration rate was severely decreased The diagnosis was ascertained by biopsy

D *Chronic renal failure* Eighteen patients had severe uraemia due to chronic glomerulonephritis (ten) and chronic pyelonephritis (eight) Many of them were in end stage renal failure and some were referred to us with oliguria of uncertain genesis

RESULTS

LDH in human kidney tissue homogenate

The average total LDH concentration and the mean distribution of the five isoenzymes in four different zones of the human kidney are shown in Table I All isoenzymes were easily identified in all tissue samples But, as appears from the calculated MSU value a significant difference in the relative distribution was observed between the single zones

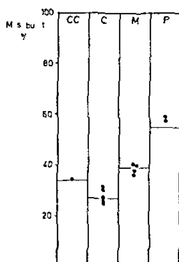


Fig 1 The relative M subunit concentration in 21 samples of kidney tissu from cortex corticis (*CC*) cortex (*C*) medulla (*M*) and papilla (*P*) The horizontal lines indicate the mean value of each group

The single values are presented graphically in Fig 1 The lowest MSU values were obtained in the cortical zone and the M subunit showed an increasing predominance when moving from the cortex towards the papilla Surprisingly the MSU value of the cortex corticis zone turned out to be significantly higher than that of the underlying cortical zone ($P < 0\ 005$) whereas no difference was observed between cortex corticis and medulla ($P > 0\ 05$) This feature hitherto not recognized will be further commented on in the discussion

LDH in serum

A The LDH concentration was elevated in all patients with *acute renal failure* and in most of them the values were high (above 100 units/ml) (Table II) The variation observed within the single group as well as between the groups may be somewhat exaggerated by the fact that values obtained do not represent true maximal concentrations as patients were received at various intervals from the onset of renal failure and the concentration has been shown to fall rapidly within the first week (14) It was repeatedly observed that serum LDH fell continuously uninterrupted by further traumatizing therapeutic or complicating events in the course of the disease Neither could we detect any influence of rapid change in the serum urea level provoked by haemodialysis The typical course in two severe cases appears from Figs 2 and 3

The concentration of all five *isoenzymes* was often markedly increased The distribution of the calculated MSU values was significantly higher than that of the normal material ($P < 0\ 001$) From the uniform distribution of the isoenzymes it appears that the high MSU values were not dependent on the increase of any single isoenzyme especially the fifth which might be liberated from e g the liver To test this possibility we correlated serum GPT to LDH 5 and the MSU value As seen from Fig 4 no correlation was observed High values of LDH 5 and

Table II The concentration of lactic dehydrogenase and the relative distribution of isoenzymes in normal persons and patients with renal diseases

	n	Total LDH (units/ml)	LDH isoenzymes ( ) I	II	III	IV	V	M subunit ( )	*P*
Controls	30	16 (7–21)[a]	23±6 0[b]	41±5 8	24±5 0	5±4 5	8±4 3	34± 6 0	
Acute renal failure	44	*120 (2 –676)*	*20*	*26*	*20*	*14*	*20*	*47± 9 8*	<0 001
I Posttraumatic	7	217 (84–676)	21	25	19	14	21	47± 9 8	
II Gastro intestinal tract	8	91 (50–149)	19	26	21	1?	22	47±14 7	
III Biliary tract	*7*	*33 (22–46)*	*15*	*30*	*1*	*14*	*19*	*48± 8 1*	
IV Other surgical cases	7	87 (50–160)	22	26	22	12	17	44± 6 2	
V Infections	9	161 (51–326)	21	23	19	17	21	48± 9 4	
VI Other medical cases	*6*	*126 (41–270)*	*21*	*28*	*17*	*14*	*20*	*46±17 0*	
Renal trauma	8	166 (31–305)	25	28	21	12	13	40± 5 7	0 0– $p > 0\ 01$
Acute glomerulonephritis	4	42 (28–53)	17	30	21	16	16	47± 1 5	<0 001
Chronic glomerulonephritis	10	29 (13–55)	24	31	22	12	11	38± 7 7	n.s.
Chronic pyelonephritis	*8*	*24 (17–30)*	*17*	*26*	*19*	*18*	*21*	*50±12.2*	<0 001

[a] Mean value and range in brackets
[b] Mean value ± one standard deviation

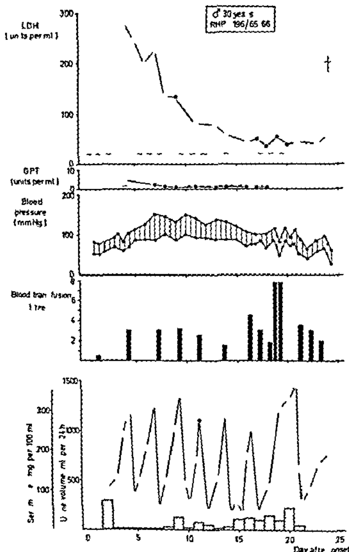


Fig 2 Serum lactic dehydrogenase (*LDH*) and glutamic pyruvic transaminase (*GPT*) correlated with the clinical course in a patient with acute renal failure following severe pancreatitis Haemodialysis was performed seven times The steep rise of the serum urea concentration between each haemodialysis reflects the dramatic course dominated by severe intra abdominal necroses and gastrointestinal bleeding

MSU were often seen despite normal or only slightly elevated GPT and even high GPT concentrations were not accompanied by any remarkable increase of the other variables

The most remarkable finding however was the almost identical average MSU value of around 47% obtained in *all* six subgroups of patients The observed values are thus apparently independent of the precipitating causes leading to renal failure and of the great variation in the maximal LDH concentrations recorded This fact further speaks in favour of a common origin of LDH as will be discussed later

B *Renal traumata* In these patients with documented lesion of renal tissue the maximal LDH concentration was well correlated with the degree of cell damage In five patients with severe uni or bilateral necroses in the kidney the LDH concentration was extraordinarily high (max LDH = 305 300 227 210 and 150 units per ml) In one patient multiple microscopic cortical infarctions were found (max. LDH = 97 units per ml) Two patients survived from minor and localized renal lesions (max LDH = 42 and 31 units per ml) The LDH concentration showed the same uninterrupted fall during the course of the disease as seen in acute renal failure

Although not significantly different from the normal the high mean MSU value of the group may be somewhat misleading as in fact only the two patients in whom renal failure compli

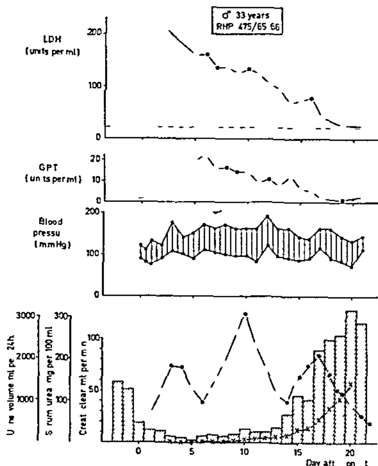


Fig 3 Serum lactic dehydrogenase (*LDH*) and glutamic pyruvic transaminase (*GPT*) correlated with the clinical course in a patient who recovered from acute renal failure following bilateral thromboendarterectomy of the femoral arteries Peritoneal dialysis was performed twice From the 7th day the patient developed progressive necroses in both legs necessitating bilateral femoral amputation on the 16th day

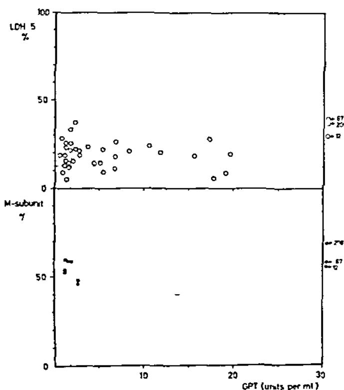


Fig 4 Relationship between the serum concentration of glutamic pyruvic transaminase (*GPT*) and the relative concentration of the fifth isoenzyme (*LDH 5*) and the M-subunit The dashed lines indicate upper normal limits As is evident from the figure no correlation exists

cated traffic accidents had significantly elevated MSU values (45 and 52%) In the other six patients values were near normal (mean = 37.6%)

C In patients with *acute oliguric glomerulonephritis* the serum LDH concentration was moderately increased (Table II) The isoenzyme pattern however turned out to be identical with that of acute renal failure Despite the small number of patients this observation is probably reliable as the standard error of the average MSU value is very low (S.E. = 0.8) The mean MSU value differed in a highly significant degree from that of the normal material ($P < 0.001$)

D In 14 of the 18 patients with *chronic renal failure* serum LDH was slightly elevated The isoenzyme patterns were indeed very inconclusive as is evident from the standard deviations However when the average MSU value of chronic glomerulonephritis is compared with that of chronic pyelonephritis a systematic difference arises although only significant at the 5% level ($0.05 > P > 0.025$)

DISCUSSION

Human kidney tissue homogenate

The presence of five isoenzymes in the kidney was described in rats by Wieme and Van Maercke (39) Our results which show an increasing relative concentration of MSU from the cortex towards the papilla confirm earlier observations in rats (28) and in rabbits (34) In human kidney Mattenheimer found MSU values corresponding to ours (calculated from data cited by Ringoir (29))

This shift from an aerobic to an anaerobic isoenzyme pattern corresponds well with the demonstration of a falling pO gradient over the kidney (2 21) Kean et al (12) demonstrated a significantly higher rate of anaerobic glycolysis in the inner medulla and a correspondingly higher oxygen consumption in the cortex Significantly decreasing blood flow rates over the three zones were demonstrated by Thorburn et al (33) Thus the MSU values are well correlated with the type of metabolism in agreement with the oxygen tension theory

The significantly higher MSU values in the cortex corticis zone than in the underlying cortical zone was therefore most unexpected We do not believe this could be due to an artefact since the site of excision was very well defined and the standard error of the average MSU value from different kidneys was small (S.E. = 0.99) Values may be somewhat disfigured by the fact that samples were taken from kidneys at autopsy but this would probably affect all zones and therefore does not influence the relative proportion between the values We have not been able to find any account of this observation in the literature but a tendency is apparent from the data given by Thorling and Jensen (34) on LDH isoenzymes in different zones of rabbit kidneys While the mean MSU value showed a linear decrease from the papilla over the inner and outer medulla to the inner cortex zone the MSU value in the *outer* cortex zone turned out to be slightly *higher* than in the *inner* cortex zone (21.1% and 19.9% respectively) Referring to our results this slight increase may be due to a high MSU value in the cortex corticis zone That they did not obtain an even higher MSU value is no doubt due to the fact that their outer cortex samples included tissue from both zones

Our results receive support from data on renal blood flow measured by Ladefoged (17) Renal blood flow in the most superficial part of the kidney as calculated from the disappearance curve of β ray emission of ^{133}Xenon was significantly lower than cortical blood flow rate calculated from the disappearance curve of the γ ray emission of ^{133}Xenon which also reflects deeper parts of the kidney His data were confirmed by autoradiography revealing a considerably delayed washout of ^{133}Xenon in a peripheral cap closely corresponding to the cortex corticis zone

A correlated study of divided blood flow and isoenzyme pattern in the two zones is in progress

The similarity of isoenzyme pattern and blood flow in the cortex corticis and the medulla of the kidney may morphologically and functionally be correlated with the presence of distal tubules in both zones

In conclusion the isoenzyme pattern of the cortex corticis zone apparently does not disprove the oxygen tension theory

LDH and isoenzymes in serum

In *chronic renal failure* the degeneration of renal tissue is usually slow and measurable liberation of total LDH from the kidney would be expected only in acute exacerbations or active infections

Almost all values were normal or borderline in this material and so were the results of Ringoir (29) West and Zimmermann (36) found elevated LDH in 43 of their 71 patients LDH was not correlated to the degree of azotaemia Jahnecke et al (9) did not detect any rise of LDH

As our material consists of patients with normal and moderately elevated serum enzyme concentrations the considerable spread of MSU values is not surprising Yet the high mean value of MSU in pyelonephritis and the low mean value in glomerulonephritis corresponds well with the site of lesion and the isoenzyme pattern in the medullary and the cortical kidney zones an observation also made by Ringoir (29)

The elevated serum LDH in *acute oliguric glomerulonephritis* and more so in *renal trauma* indicates altered membrane permeability and/or necrosis of renal tissue Considering the slight difference between the MSU value in normal serum and the kidney zones involved a significant change of the isoenzyme pattern in serum could hardly be expected Accordingly normal values of MSU were found in six of the patients with renal trauma while surprisingly two of the traumatic patients and all four patients with acute glomerulonephritis showed elevated values This may be due either to extrarenal lesions or to adaptive change of renal LDH synthesis as discussed below

The high LDH concentration and the elevation of all five isoenzymes in serum from patients with *acute renal failure* confirm our previous findings (14) and have also been observed by others (9 29 36)

The important question arises to what extent is LDH liberated from *extrarenal* tissues owing to cell necrosis and/or shock induced hypoxia in the tissue during the preceding events leading to acute renal failure

The elevated concentration of LDH 1 and 2 might result from severe haemolysis or myocardial infarction This probably explains the low MSU value of 29% in one patient with acute renal failure following severe haemolysis (haemolytic crisis) Otherwise none of the patients presented evidence of preceding myocardial infarction or haemolysis

The dominant and uniform increase in the concentration of M subunits throughout the material might be due to liberation of LDH from the liver and muscles considering the high concentration of LDH 5 (and 4) in these tissues A high venoarterial difference of LDH concentration in *hepatic femoral* and *renal* vessels has been demonstrated in dogs which succumbed from *sustained deep* and *irreversible* haemorrhagic shock (35)

However in many of our patients no established shock was recorded and deep protracted shock was rare and never irreversible in the initial phase Hypotensive crises during the course of renal failure or irreversible shock leading to death did not provoke a second rise of the LDH concentration (Fig 2) Similarly extensive necroses in both legs eventually leading to bilateral femoral amputation in one patient did not interrupt the continuous fall in the concentration of LDH (Fig 3) In experimental endotoxin shock LDH and glutamic oxalacetic transaminase (GPT) both present in high concentrations in the kidneys were considerably elevated while creatine phosphokinase (muscle) was unaltered (15) Furthermore serum ornithyl-carbamyl transferase (liver) and creatine phosphokinase (muscle) were within normal limits despite a marked increase of LDH 5 in patients with acute renal failure studied by Ringoir (29) Yet it is a common clinical experience that some patients with acute renal failure are slightly icteric despite the lack of evidence of infectious or toxic affection of the liver This so-called shock liver may give rise to some elevation of GPT But with few exceptions the majority of our patients showed either normal or only moderately elevated GPT concentrations (< 10 units per ml) and there was no correlation with either the *relative* or the *absolute* concentration of LDH 5 or M subunit in our material Furthermore the high MSU value was certainly not dependent on any prominent or isolated elevation of LDH 5 which might derive from the liver In individual patients with extensive traumatic cerebral necroses we recently observed moderately increased concentrations of LDH (max 71 units/ml) with a rather uniform distribution on the five isoenzymes However as none of our patients showed autopsic or clinical signs of cerebral lesions we consider this source improbable in our material

In conclusion we believe that LDH is liberated to some extent from extrarenal tissue especially the liver in acute renal failure On the other

hand this can only explain a minor part of the total concentration of LDH and, with the exception of a few special cases there is no evidence of any significant influence on the M subunit concentration recorded

We must therefore assume that LDH is mainly liberated from the *kidney* and that the isoenzyme pattern and hence the MSU value is determined by this source The kidney has a very high concentration of LDH and considering its isoenzyme pattern it is the only single organ that might cause the observed isoenzyme pattern in serum

Liberation of LDH from the kidney has been demonstrated in experimental haemorrhagic shock (13 35) and selective renal infarction or ischemia in experiments on dogs was followed by a prompt elevation of plasma LDH together with a more gradual increase of LDH in urine (3) Elevated LDH in urine has been observed in acute renal failure in man (31) Similar unpublished observations were frequently made in connection with our material and urine isoenzyme electrophoresis often showed elevation of all five fractions Furthermore the observation of an almost identical MSU value in the six pathogenetically different subgroups and the continuous fall in total LDH concentration in serum uninterrupted by additional traumatizing complications strongly indicates a single dominant source which for the reasons stated above must most evidently be the kidney

The isoenzyme pattern

When the high MSU values obtained in *acute renal failure* and *acute oliguric glomerulonephritis* are compared with those of the different zones in the kidney *it becomes apparent that one* would have to assume the existence of a lesion mainly located in the inner medulla and the papilla This is quite inconceivable from a morphological and functional point of view Maintaining that a significant extrarenal contribution of M subunits can generally be excluded we can therefore only assume that the renal LDH has been subjected to some modification before it is liberated to the serum

Accordingly we have advanced the hypothesis that the synthesis of LDH in kidney cells is *adapted* for a preferential production of the M subunits in acute renal failure (25) This adaptation probably takes place within the first 24–48 hours and is mainly restricted to the renal cortex. The adequate stimulus for this adaptation is assumed to be *low oxygen tension* in the cortical tissue during the initial phase of acute renal failure

The evidence of differential synthesis of H and M subunits in cells according to the prevailing oxygen tension has been presented above *Adaptive* change of LDH synthesis within the same tissue provoked by a change of oxygen tension has frequently been observed in vitro (4 8) and in vivo (6 7) That a demonstrable adaptation of the LDH synthesis in vivo is possible within 24 hours has been shown by Thorling and Jensen (34) who submitted rabbits to low oxygen tension for 24 hours and obtained a significant increase of the M subunit concentration in the renal cortex the zone most susceptible to hypoxia Finally direct evidence in support of our hypothesis of adapted LDH synthesis in the renal cortex in acute renal failure has recently been provided by the data reported by Ringoir (30) showing a significant increase of the M subunit concentration in renal biopsies in the acute stage of the disease His conclusion from these data was similar to ours

It has been much disputed whether *low oxygen tension* is present in renal tissue in the acute oliguric state As shown by Thaysen et al (32) the normal renal cortex has a high oxygen consumption which can be divided into a main functional (or suprabasal") fraction covering the energy consumed by the active sodium absorption in the proximal tubules and a small basal fraction to cover the oxydative metabolism of the cells under basal conditions i e in the non *absorbent state* *This basal oxygen consumption* amounts to 1 μmol O /g/min and hypoxia is only present when this oxygen supply is not available In acute renal failure renal blood flow has been shown to be reduced to about one third of normal (24) Oxygen consumption is proportionately reduced as arteriovenous oxygen extraction is rather constant down to a certain lower limit of renal blood flow (16 22 24) Thus this oxygen supply must be regarded as sufficient to cover basal metabolism in the non absorbent kidney and can hardly condition an adequate lowering of oxygen tension to provoke an adaptive change of LDH synthesis

However in the very early phase of acute

renal failure Pedersen et al (26 27) succeded in measuring renal blood flows down to *one sixth* of normal and other available data on arterio-venous oxygen differences with such low blood flows (16) make it highly probable that true hypoxia exists after all This acute hypoxia may account for the excessive release of intracellular LDH owing to impairment of cellular integrity (not necessarily cell necroses) as well as the adaptive change of LDH synthesis observed

Although very few data exist on renal metabolism in acute oliguric glomerulonephritis it is tempting to draw a parallel between the serum isoenzyme pattern in that condition and that of acute renal failure Cargill and Hickam (5) observed a highly significant reduction of oxygen consumption in acute glomerulonephritis which was *closely correlated with the decrease of the* glomerular filtration rate (GFR) and Ladefoged (17) measured decreased renal blood flow in subacute glomerulonephritis similarly correlated with the decrease of GFR Recently he found the renal blood flow decreased to 0 6 ml/g/min in a patient with *renal failure* due to *acute* glomerulonephritis This value is of the same order as those obtained in the initial phase of acute renal failure GFR was close to zero in our patients and it may be assumed that true hypoxia was present in the acute oliguric state thus accounting for the high serum concentration of the M subunit in this pure renal disease

CONCLUSION

The results of this study are in agreement with the concept of a low oxygen tension due to reduced renal blood flow in *acute renal failure* Although they do not allow any conclusions as to the pathogenesis of the initial shut-down of renal function they suggest the following sequence of events

1 In the initial phase the integrity of renal cells is impaired by the acute hypoxia, resulting in a massive release of presumably unmodified intracellular LDH

2 In the course of one or two days (perhaps hours) LDH synthesis is gradually adapted to the hypoxic state evidenced by the relative increase of the M subunit in renal tissue and serum

3 Basal energy metabolism is gradually re established possibly based in part on anaerobic glycolysis Hence the cellular integrity recovers and the liberation of LDH ceases as seen from the gradual decline of serum concentration

To support this hypothetical model many features still have to be elucidated Enzyme analysis in tissue and serum correlated with other parameters may in future investigations throw further light especially on the acute phase of the disorder

REFERENCES

1 Appella, E & Markert C L Dissociation of lactate dehydrogenase into subunits with guanidine hydrochloride Biochem Biophys Res Commun 6 171 1961

2 Aukland K & Krog J Renal oxygen tension Nature (Lond) 188 671 1960

3 Bett M M Skaggs J D Johnston Gloria & Hershey F B Lactic dehydrogenase activity of dog plasma and urine following renal injury Surg Forum 9 65 1959

4 Cahn R D Cellular damage and the control of lactic dehydrogenase synthesis in cell cultures by oxidative metabolites J Cell Biol 19 1? A 1963

5 Cargill W H & Hickam J B The oxygen consumption of the normal and the diseased human kidney J Clin Invest ?8 5 6 1949

6 Dawson D M Goodfriend T L & Kaplan N O Lactic dehydrogenases functions of the two types Rates of synthesis of the two major forms can be correlated with metabolic differentiation Science 143 9?9 1964

7 Goldman R D Kaplan N O & Hall T C Lactic dehydrogenase in human neoplastic tissues Cancer Res 24 389 1964

8 Goodfriend T & Kaplan N O Induction of changes in the subunit composition of lactic dehydrogenase J Cell Biol 19 28 A 1963

9 Jahnecke J Loffler G Meisch M & Streicher E Beitrag zur Pathogenese des akuten Nierenversagens Untersuchungen uber Lactatdehydrogenase Isoenzyme Klin Wschr 45 466 1967

10 Jensen K A & Laursen, T LDH isoenzymes Separation on agarose and spectrophotometric scanning of the isoenzymes Scand J clin Lab Invest Suppl 86 155 1965

11 Kaplan N O & Goodfriend T L Role of the two types of lactic dehydrogenase In Advances in enzyme regulation vol II pp ?03-212. Pergamon Press Oxford 1964

12 Kean E L Adams Patricia H Winters R W & Davies R E Energy metabolism of the renal medulla Biochim biophys Acta 54 474 1961

13 Kemp E & Laursen T On the renal release and excretion of enzymes in experimental nephritis and shock Acta path microbiol scand 54 85 1962

14 Kemp E Lange H Laursen T & Nielsen V K Elevated serum enzyme activity in acute renal failure In Proc Europ Dial Transpl Ass 1st Congr pp 135-139 Scheltema and Holkema, Amsterdam 1964

15 Konttinen A Rajasalmi M & Paloheimo J Serum enzyme activities in endotoxin shock Amer J Physiol 07 385 1964
16 Kramer K & Deetjen P Beziehungen des O Verbrauchs der Niere zu Durchblutung und Glomerulusfiltrat bei Änderung des arteriellen Druckes Pflugers Archiv ges Physiol 271 782 1960
17 Ladefoged J Personal communication 1967
18 Laursen T & Hansen P F A fluorimetric method for measuring the activity in serum of the enzyme glutamic pyruvic transaminase Scand J clin Lab Invest. 10 53 1958
19 Laursen T A fluorimetric method for measuring the activity in serum of the enzyme lactic dehydrogenase Scand J clin Lab Invest. 11 134 1959
20 Laursen T & Nielsen, V K Quantitative determination of lactic dehydrogenase isoenzymes To be published
21 Leonhardt, K O and Landers R R. Oxygen tension of the urine and renal structures New Engl J Med 269 115 1963
2 Levy M N Influence of variations in blood flow and of dinitrophenol on renal oxygen consumption Amer J Physiol 196 937 1959
23 Markert C L Lactate dehydrogenase isoenzymes Dissociation and recombination of subunits Science 140 13 9 1963
24 Munck O Renal circulation in acute renal failure Diss Blackwell, Oxford 1958
25 Nielsen V K Adaption of the synthesis of lactic dehydrogenase to hypoxia in acute renal failure In Actual problems in clinical biochemistry (eds H Aebi H Mattenheimer & E Schmidt) Huber Bern-Stuttgart 1968 In print
26 Pedersen F., Baunde B O Berthel en H C Christiansen P Kemp E Ladefoged J & Winkler K Renal blood flow and mean circulation time for red cells and plasma in acute renal failure In Renal failure and replacement of renal function (ed. D N S Kerr) pp 77–81 Excerpta Medica Foundation Amsterdam 1965
7 Pedersen F Ladefoged J Winkler K., Baunøe B & Munck, O Renal blood flow and circulation times in acute toxic nephropathy following poisoning with arsine (AsH_3) Ugeskr Læg 1 9 555 1967
28 Richterich R Schafroth P & Franz, H E Das isolierte Glomerulum der Rattenniere III Heterogenität der Lactat Dehydrogenase in Nierenrinde Nierenmark und Glomerulum Enzymol biol clin I 114 1961–6?
9 Ringoir S Toepassing van de Gedifferentieerde LDH Bepaling Bij Experimentele en Klinische Niereandoeningen Diss Arscia, Brussels 1967
30 — Correlations between kidney serum and urinary enzyme activity In Actual problems in clinical biochemistry (eds H Aebi H Mattenheimer & E Schmidt) Huber Bern-Stuttgart In print
31 Rosalki S B & Wilkinson, J H Urinary lactic dehydrogenase in renal disease Lancet ? 3?7 1959
3 Thaysen J H Lassen N A & Munck, O Sodium transport and oxygen consumption in the mammalian kidney Nature 190 919 1961
33 Thorburn, G D Kopald H H Herd J A., Hollenberg, M., O Morchoe C C C & Barger A C Intrarenal distribution of nutrient blood flow determined with Krypton[85] in the unanesthetized dog Circulat Res 13 290 1963
34 Thorling E. B & Jensen, K. The lactate dehydrogenase isoenzyme in various organs of the rabbit in anaemia hypoxia and after cobalt administration With special reference to changes in the isoenzyme pattern in the kidney cortex Acta path microbiol scand 66 4 6 1966
35 Vesell E S Feldman M P & Frank E D Plasma lactic dehydrogenase activity in experimental hemorrhagic shock Proc Soc exp Biol (N Y) 101 644 1959
36 West M & Zimmerman H J Serum enzymes in disease IV Lactic dehydrogenase and glutamic oxalacetic transaminase levels in renal disease J Lab clin Med 5? 185 1958
37 Wieland T & Pfleiderer G Chemical differences between multiple forms of lactic acid dehydrogenases Ann N Y Acad Sci 94 691 1961
38 Wieme R. J An improved technique of agar gel electrophoresis on microscope slices Clin chim. Acta 4 317 1959
39 Wieme R J & van Maercke Y The fifth (electrophoretically slowest) serum lactic dehydrogenase as an index of liver injury Ann N Y Acad Sci 94 898 1961
40 Wroblewski F The clinical significance of lactic dehydrogenase activity in the milieu interieur Scand J clin Lab Invest Suppl 31 230 1957

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THE EFFECT OF NICOTINIC ACID ON THE DIURNAL VARIATION OF THE FREE FATTY ACIDS OF PLASMA

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Abstract In four patients with hyperlipemia the diurnal variations of plasma FFA were studied before and during nicotinic acid treatment It was found that the mean plasma FFA level was higher during than before treatment with nicotinic acid although a reducing effect on the plasma triglycerides and plasma cholesterol concentrations was observed The hypothesis that the lowering effect of nicotinic acid on the plasma lipids is secondary to an inhibition of the FFA mobilization from adipose tissue is, reasonably not valid

It has long been known that long term nicotinic acid administration exerts a lowering effect on plasma cholesterol and total fatty acids (1 7) It was subsequently shown by Carlson and Oro (3) in acute experiments that nicotinic acid depressed the level of plasma free fatty acids (FFA) and Carlson (2) demonstrated that this was due to an inhibition of the lipolysis in adipose tissue Carlson and Oro (3) suggested that the effect on other plasma lipids might be secondary to a reduced transport of fatty acids from the adipose tissue to the liver However the lowering of FFA by nicotinic acid was followed by a rise exceeding the original level The duration and extent of this increase of FFA was not studied in detail Recently this phenomenon was studied after intravenous injection of nicotinic acid (9) A very impressive increase of FFA was noted between 2 and 4 hours after the administration of nicotinic acid

The present study was started in order to elucidate the effect of therapeutic doses of nicotinic acid on the diurnal variation of plasma FFA Frequent recording of the plasma FFA level was feasible with a capillary method for the analysis of FFA (6) which gives results close to the arterial level

METHODS

FFA was determined according to Laurell and Tibbling (6) plasma was separated from the blood samples within 10 min. Triglycerides were determined according to Laurell (5) and cholesterol according to Ness et al (8) The patients were given a highly standardized diet, containing 63 g of fat, 104 g of protein and 158 g of carbohydrates daily on both admissions (4) Breakfast served at 8 30 a.m., luncheon at 12 a.m and dinner at 5 p.m. No other food was allowed.

Nicotinic acid (Nicangin®) tablets were administered at 8 a.m 2 p m and 9 p m

CASE REPORTS

Case 1

Woman, aged 41 two years previously myocardial infarction, when elevation of plasma cholesterol value was observed Blood samples from relatives were not analysed as they live outside Sweden She was admitted in December 1966 when the first examination in the present study was made After this (first) admission she was given nicotinic acid (Nicangin®) 0.5 g three times daily for about one month and was then reexamined Owing to side effects of the drug the patient refused to take the full, recommended therapeutic dose

Case

Man aged 42, suffered from pectoral angina for about three years His mother and two of her brothers had died aged about 50 of myocardial infarction.

For the present study the patient was first examined in November 1966 when hypercholesterolemia was diagnosed. He was then given nicotinic acid (Nicangin®) in increasing doses up to 1 g three times daily before reexamination about two months later Only slight side effects of the drug were noted

Case 3

Man, aged 56 myocardial infarctions in 1965 and 1966 Hypercholesterolemia and hypertriglyceridemia were diagnosed in 1966 In the present study the patient was first examined in June 1967 No heredity traits of interest were observed

Table I *Plasma lipids before and during treatment with nicotinic acid*

Nicotinic acids – = before treatment + = during treat ment *Cholesterol and triglycerides fasting value FFA mean value calculated from the intergrated diurnal curves*

Case no	Nicotinic acid	Cholesterol (mg/100 ml)	Tri glycerides (mM)	FFA mean (mEq/l)
1	–	376	1 26	0 38
1	+	296	0 63	0 92
2	–	446	0 68	0 43
2	+	340	0 47	0 67
3	–	460	4 34	0 57
3	+	400	2 12	0 62
4	–	512	0 85	0 77
4	+	412	0 72	1 09

Nicotinic acid (Nicangin®) was then given in rising doses up to the full dose of 1 g thre times daily About one month later he was reexamined

Case 4

Man aged 27 known to have essential familiar hyper cholesterolemia since 1966 Admitted for the present study in March 1967 After this (first) examination pa tient was given nicotinic acid (Nicangin®) in increasing doses up to 1 g three times daily without any troublesome side effects He was re xamined about two months later

RESULTS

As shown in Table I the mean level of FFA dur ing 24 hours was not depressed in any of the pa tients but strikingly elevated in the majority dur ing administration of nicotinic acid The mean increase was 0 29 mEq/l Nonetheless both tri glycerides and cholesterol were effectively de

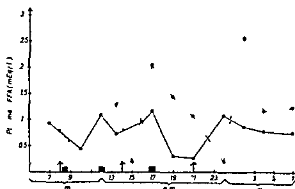


Fig 1 Diurnal plasma FFA level in case 4 without nicotinic acid (*unbroken line*) and during administration of nicotinic acid (*broken line*) The nicotinic acid tablets were given at times indicated by arrows and the meals were served at times indicated by squares

pressed (Table I) During daytime (Table II and Fig 1) an FFA depressing effect of nicotinic acid alternated with periods of increased FFA concen tration The net result of nicotinic acid on FFA level during daytime is rather slight On the other hand all the patients showed a striking increase in FFA during the night

DISCUSSION

The alternatively depressing and increasing effect of nicotinic acid on the FFA level corresponds to the findings after a single dose (3 9) As the in creasing effect is strikingly dominant during the night the total result is an increase of the mean FFA level during 24 hours on administration of nicotinic acid in the recommended therapeutic

Table II *The diurnal variation of plasma FFA*

0 = without treatment Nic = during treatment with nicotinic acid Somewhat different sampling times were used for cases 1 2 and 3 4

	FFA (mEq/l)								
	Case 1		Case 2			Case 3		Case 4	
Time	0	Nic	0	Nic	Time	0	Nic	0	Nic
7 a.m	0 54	0 83	0 81	0 47	7 a m	0 70	0 70	0 94	1 05
9 a m	0 18	0 26	0 39	0 21	10 a m	0 33	0 19	0 44	0 32
11 a m.	0 29	0 40	0 43	0 84	12 a m	0 53	0 14	1 09	0 32
1 p m.	0 19	0 ˍ6	0 7	0 33	1 30 p m	0 40	0 59	0 73	1 31
3 p m	0 10	0 16	0 15	0 17	4 p m	0 70	0 22	0 96	0 70
5 p m.	0 52	2.50	0 83	0 40	5 p m	0 83	0 40	1 17	2 05
7 p m.	0 14	0 30	0 16	0 60	6 30 p m	0 45	0 44	0 30	1 45
9 p m.	0 17	0 68	0 22	0 36	9 p m.	0 44	0 77	0 26	1 08
12 p m.	0 76	2.30	0 32	0 27	11 30 p m	0 79	0 29	1 08	0 0
2 a.m	0 63	1 36	0 77	1 96	1 30 a m	0 69	1 57	0 86	2 58
4 a m	0 56	1 01	—	—	4 a m	0 61	0 95	0 78	1 17
7 a.m	0 54	0 93	0 47	1 10	7 a.m.	0 53	0 93	0 77	1 25

dose This is not compatible with the hypothesis that the effect of nicotinic acid on the plasma lipids is secondary to an inhibition of FFA mobilization (3) Apparently another explanation must be sought as the plasma triglycerides and cholesterol were depressed in all our patients in spite of an increase in the mean FFA level

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REFERENCES

1 Altschul R. & Hoffer A. Arch biochem 73 4 0 1958
2 Carlson, L. A Acta med scand 173 719 1963
3 Carlson L. A & Oro L. Acta med. scand 17_ 641 196_
4 Hedner P., Nordén, A., Persson, A Persson, M & Schersten, B In Portionsforpackad djupfryst diabetes- och avmagringskost p 37 Bloms Boktryckeri, Lund 1967
5 Laurell S Scand. J clin Lab Invest 18 668 1966
6 Laurell S & Tibbling, G Clin. chim Acta 16 57 1967
7 Marini M. A Nebbia M & Zanalda C Pren med argent. 46 18_5 1959
8 Ness A T., Pastewka, J V & Peacock, A C Clin chim Acta 10 2 9 1964
9 Pereira, J N J Lipid Res 8 239 1967

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PRIMARY AMYLOIDOSIS IN LUNGS AND HEART

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Abstract A case of presumably primary amyloidosis, located in lungs and heart in a 70 year-old man is reported During the last three years of his life the patient presented symptoms of congestive heart disease The diagnosis was made at post mortem microscopic examination The frequency of primary amyloidosis of lungs and heart is commented on.

Amyloid deposition in the lungs is reputed to be a rare condition in the primary as well as in the secondary form of amyloidosis The frequency is estimated somewhat differently by different authors Spencer (8) in Pathology of the Lung mentions that primary amyloidosis is an unusual finding and secondary amyloidosis very unusual

Some publications suggest that amyloid deposition in the lungs in the primary generalized form of the disease is not an altogether unusual finding in fact Rukavina et al (7) from post mortem examinations of 142 cases of primary generalized amyloidosis found the lungs to be moderately to considerably involved in 44 cases (30 9%)

Symmers (9) in a material of 145 cases of primary generalized amyloidosis found infiltration of the lungs in about 30 % of the cases

Weiss (12) mentions that the lungs are involved in nearly half of the cases of primary generalized amyloidosis

The situation differs in the cases when the amyloid deposition is totally or mainly located in the lungs

Hambach (2) reports the number of published cases of that kind not to surpass 35

Prowse (6) could collect only 18 authentic cases of amyloidosis of the lungs

Tennstedt (11) mentions that compared with other organs amyloid is found only rarely in the lungs this is especially true of the secondary form of the disease In primary amyloidosis the lungs seem to be more frequently attacked

In Denmark Noring and Paaby (5) have described a case of amyloid deposition in the deep paths of respiration and Sørensen (10) a case of tumor like amyloid deposition in the lung

In the heart amyloid deposition is seen more frequently than in the lungs Rukavina et al (7) found the heart to be involved in 92 of 142 cases (64 7%)

Symmers (9) in primary generalized amyloidosis found the heart to be involved in no less than 90% of cases

Several publications point out a relation between amyloidosis of the heart and high age Thus King (4) reports five cases of primary amyloidosis located in heart and lungs the average age was 87 8 years

Josselson et al (3) report 29 cases of primary amyloidosis with the heart as the only affected organ The average age was 82 years

In the following a case of presumably primary amyloidosis in lungs and heart is reported The diagnosis was made at microscopic examination of tissues removed post mortem without anyone previous suspicion of the disease

For this reason only tissues from a few organs were removed for histological examination and so it may reasonably be said that the examination concerning the extension of the amyloidosis has not been comprehensive enough

Despite this the case is presented partly because primary amyloidosis especially in the lungs is remarkable and partly in order to draw attention to the diagnosis of pulmonal amyloidosis as one of the possibilities to be considered when the clinical roentgenological and patho-ana

tomical findings presented by this patient are encountered

CASE REPORT

A 70-year-old married man a former mechanic was admitted to the Sundby Hospital, Medical Department on December 12, 1963 because of congestive heart disease and degeneration of cervical intervertebral disc

Prior to admission, he had been in good health and never hospitalized but in 1957 he had consulted the *medical out patient department of the Finsen Institute* The diagnoses there were neuritis nn radiales osteochondrosis art unchovertebralis myoses He was treated with aneurin injections and physiotherapy ESR was then 39 mm h, Hb 97%

He was admitted because of weakness that had grown worse through three years dyspnoea and crural oedemas Sometimes he had nocturnal fits of dyspnoea The patient denied having ever had rheumatic fever There was no information on the consumption of tobacco Moreover during the last seven years he had been suffering from paraesthesia and gradually developing pains in the three radial fingers of each hand The sensation in these fingers had decreased and wounds healed slowly The patient claimed to tolerate all kinds of food

He had stopped working three years earlier but not because of illness His own doctor had treated him with digitalis and diuretics

On admission to Sundby Hospital his general condition was found to be poor nutritional state average respiration slightly quickened there was slight cyanosis of lips and nails Physical examination of the lungs was normal the heart action was irregular with pulse deficit No abnormal heart sounds were heard The liver was palpable about one hand-breadth below the curvature there was no ascites Massive oedema of both legs There was muscular atrophy of the thenar muscles of both hands, and the three radial fingers of both hands were tapering, bluish, and weak All reflexes were normal

The height was 179 cm, weight 77.5 kg.

The ECG revealed atrial fibrillation and diphasic T waves in all leads.

X rays of the chest showed considerably enlarged breadth of the heart and increased vascularity of the lungs There was a questionable exudate in the left pleural cavity X rays of the cervical columna revealed osteochondrosis of the 5th (?) cervical vertebra

Laboratory findings

Hb 14 9 g/100 ml ESR 40 mm h, fasting blood glucose 74 mg/100 ml urine amylase 150 W units/ml, BP 140 80 the urine was without protein glucose or blood microscopy of urine was normal creatinine 1.3 mg 100 ml creatinine clearance 58 and 64 ml min.

Neurological examination

Bilateral atrophy of thenar muscles, trophic changes of hands and fingers, a positive Babinski on the left (because of age) X rays of hands and skull normal EMG of the affected finger muscles revealed neurogenic atrophy Otological examination because of hoarseness for some years revealed red and thickened but freely movable vocal chords

The patient was treated with digitalis and diuretics

During his stay at hospital the patient did not exhibit any particular symptoms He was constantly confined to his bed and sometimes he had nocturnal fits of dyspnoea, Temperature and pulse rate were normal

On January 2 1964 after having been in hospital for about three weeks he suddenly died without any previous increase of temperature or signs of pneumonia

Post mortem examination

Fibrinous coatings on both pleurae but no exudate The lungs were large and firm the consistency being nearly liver like The cut surface was red and homogenous Small pieces of lung remained floating in water The bronchial mucosa was haemorrhagic increasingly towards the periphery There was no secretion in the bronchial tree The mediastinal lymph nodes were normal The pericardium was smooth and glistening. There was no liquid in the pericardial cavity The heart weighed 680 g, measured 13 × 14 cm i.e much enlarged The right ventricle was 5 mm the left ventricle 18 mm The aortic ostium was narrowed to the size of the fifth finger and in the posterior wall of the left ventricle there were calcifications like those after rheumatic endocarditis. The mitral ostium was unchanged the myocardium considerably hypertrophic all cavities were dilated In the posterior wall of the left ventricle there was a 5 × 6 cm fibrosed area The coronary arteries and aorta presented severe arteriosclerosis The pulmonary artery and superior caval vein were unchanged

Oesophagus, ventricle and duodenum were normal

The liver measured 30 × 20 × 8 cm presenting the appearance of a nutmeg liver but the consistency was normal with no evidence of amyloidosis The spleen weighed 190 g on the cut surface the trabecular pattern was seen to be increased In the left adrenal gland a yellow brown round tumour was found measuring 2 cm in diameter There was no evidence of amyloidosis of the adrenals or kidneys

The skull was not opened The amyloid test was not done

Microscopic examination of the lung revealed considerable alterations In nearly all alveolar walls deposits of an eosinophilic amorphous material were seen, and also in the vascular walls which were nearly diffusedly infiltrated Many vascular walls were narrowed on account of this material which was seen in all layers of the vascular wall (Fig 1) In a single bronchial branch a solid accumulation of polymorphonuclear leucocytes was seen. A section, stained with methyl violet revealed that this material in some places showed signs of metachromasia Microscopic diagnosis amyloidosis primaria pulmonum, bronchitis purulenta.

A section from the myocardium disclosed very considerable interstitial fibrosis Scattered in the interstitial tissue amorphous deposits were seen which were slightly eosinophilic when stained with methyl violet they showed signs of metachromasia In some areas there were histiocytes, lymphocytes, single plasma cells and polymorphonuclears. Close to areas with the amorphous material



Fig 1 Lung showing amyloid deposits in alveolar and vascular walls HE ×125



Fig 2 Myocardium showing interstitial deposits of amyloid and foreign body giant cell HE ×125

foreign body giant cells were seen (Fig 2) Microscopic diagnosis amyloidosis primaria myocardii (with foreign body reaction) fibrosis interstitialis myocardii

In one adrenal gland a cortical adenoma was demonstrated

The spleen revealed stasis otherwise no pathological changes

Microscopy of skin and medulla spinalis did not disclose any pathological changes

COMMENT

Amyloidosis was diagnosed at microscopic examination of the lungs and myocardium

As mentioned above the finding was unexpected as during his period of illness the patient had never been suspected of having amyloidosis At post mortem examination the only grossly remarkable findings were the considerably hypertrophied heart with evidence of earlier endocarditis and the large firm lungs being nearly liver like in consistency Based upon clinical and post mortem findings the patient was found to have been suffering from the following recognized diseases

Endocarditis with later development of aortic stenosis and considerable hypertrophy of the left ventricle Presumably the patient had had rheumatic fever many years previously though this was denied However according to the literature there is no basis for supposing any connection between rheumatic fever and amyloidosis

Osteochondrosis

An about 7 year-old neurologic disease manifesting itself by finger paraesthesiae and neurogenic atrophy of finger muscles

Purulent bronchitis presumably terminal since the patient had not been particularly annoyed by coughing previously

None of these diseases may reasonably be suspected of leading to amyloidosis

Did the patient have multiple myeloma? Unfortunately no examination was made of the serum proteins or of the bone marrow since the diagnosis of multiple myeloma was not suspected clinically For the same reason no post mortem bone marrow examination was done

The localization of amyloid in lungs (and myocardium) corresponds with that expected in the primary form of amyloidosis as mentioned above secondary amyloid deposition in the lungs is said to be very rare although it may be found

In agreement with this is the lack of amyloid deposition in the organs generally involved in secondary amyloidosis kidney liver spleen adrenal gland Indeed no histological examination was made of kidney and liver but at gross examination these organs were not suspected of amyloidosis

On the other hand there is no evidence suggesting the presence of any amyloid deposition in the tongue which would have been a very characteristic feature in primary amyloidosis

The histological finding of giant cells in the *myocardium points to primary amyloidosis* as this is a rare finding in the secondary form (11)

Moreover it must be mentioned that atypical or even negative staining reactions as in this case are more common in primary than in secondary amyloidosis

When comparing symptoms with post mortem findings the increasing cardial insufficiency is found to be in agreement with the considerably enlarged heart the left ventricle of which especially because of the aortic stenosis was hypertrophic The severe interstitial fibrosis and the amyloid deposits have further contributed to the cardiac insufficiency

The patient s dyspnoea was probably due to stasis as well as to the extensive amyloid deposits in the vascular walls of pulmonary vessels and alveolar walls

The hypertrophy of the right ventricle must probably be put down to the pulmonary changes

For the clinicians it was natural to interpret the condition as an ordinary although severe cardiac insufficiency and none of the patient s symptoms pointed to amyloidosis nor did the chest X ray pictures which revealed increased vascular patterns, as in stasis

Compared with earlier published cases we have found this case to be most like that of Ferris (1) In his 52 year-old patient the clinical course was characterized by several years of dyspnoea and chronic bronchitis Post mortem examination revealed nodular amyloid deposits in the vascular walls of pulmonary vessels in alveolar walls and in the bronchioles In the heart the myofibrils were found to be surrounded by amyloid which again was found in the vascular walls

In addition our case is much like that of Hambach (2) whose *post mortem examination* of a 87 year old woman who for years had been suffering from a moderate dyspnoea revealed amyloid deposition in the lungs partly diffusedly located in vascular walls partly tumour like Moreover small quantities of amyloid were present in myocardium spleen and liver

REFERENCES

1 Ferris H W Amyloidosis of lungs and heart. Amer J Path 12 701 1936
2. Hambach R. Die primäre Lungenamyloidose Zbl. allg Path path Anat. 104 424 196?
3 Josselson A T Amyloid localized to the heart. Analysis of 29 cases Arch Path 54 359 196?
4 King, L. S Atypical amyloid disease with observations on a new silver stain for amyloid. Amer J Path 24 1095 1948
5 Noring, O & Paaby H Diffuse amyloidosis in the lower air passages Acta path microbiol scand 31 470 1952
6 Prowse B C Thorax 13 308 1958
7 Rukavina, J G Primary systemic amyloidosis, a review and an experimental, genetic and clinical study of 29 cases with particular emphasis on the familiar form Medicine (Baltimore) 35 239 1956
8 Spencer H Pathology of the lung p 548 Pergamon Press New York 1962.
9 *Symmers W S Primary amyloidosis a review* J Clin Path 9 187 1956
10 Sörensen H R. Primary isolated nodular amyloidosis of the lung. Acta chir scand Suppl .83 16., 1961
11 Tennstedt A Zur Kenntnis des isolierten Lungen amyloids Frankfurt. Z. Path 68 205 1957
12. Weiss L Isolated multiple nodular pulmonary amyloidosis Amer J Clin Path. 33 318 1960

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DEATH FROM ARTERIOSCLEROTIC HEART DISEASE OUTSIDE HOSPITALS

A Study of 2678 Cases in Stockholm with Particular Reference to Sudden Deaths

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Abstract During the years 1955 1960 1964 1965 and 1966 678 deaths outside the hospitals in the Stockholm region du to arteriosclerotic heart disease (ASHD) were certified by autopsy at the Department of Forensic Medicine Karolinska Institutet, Stockholm Of these 1 13 deaths were considered sudden Owing to detailed pol ce record and autopsy protocols the place and ac tivity at time of death as well as postmortem findings could be studied In the males below the age of 70 death at work was found to o cur less frequently than expected as calculated from the estimated time spent there Heavy physical exercise and mental stress together were found to pre ede no more than 5 per cent of the male sudden deaths and only a minority of the female Autopsy revealed a recent myocardial infarction in 34% of the males below the age of 50 and in about 20% of the older males Even lo er figures were recorded in the female Possible causes of these apparently infrequent f nd ngs are discussed In agreement with the observations of other authors an overrepresentation of myocardial ruptures was found in the females Previous symptoms suggest ve of ASHD were considered absent in 37% of the male sudden deaths below the age of 50 and in about 0% of the older subjects of both sexes

There is cumulating evidence of a high mortality within the first hours after the onset of an acute coronary event Consequently a high proportion of early deaths from arteriosclerotic heart disease (ASHD) occur outside the hospitals and are medically unattended

The exact characteristics of deaths from ASHD outside the hospitals in a given community such as sudden deaths have not been possible to re cord *Autopsies carried out by Medical Examiners* or Coroners provide some data on deaths outside the hospitals ascribed to ASHD but studies based on their files have been limited primarily to demographic characteristics that have been noted on the death certificate (6)

In view of re ent advances in cardiac resuscita tion and treatment of serious arrhythmias avail able data on deaths from ASHD outside the hospitals may serve as an orientation when plan ning measures for reducing the death toll of ASHD The present study deals with autopsied deaths from ASHD which have been admitted to the Department of Forensic Medicine Karo linska Institutet Stockholm during five years 1955 1960 1964 1965 and 1966

MATERIAL AND DEFINITIONS

During the years 1955 1960 1964 1965 and 1966 a total of 2678 deaths (1819 males and 859 females) as cribed to ASHD (WHO list diagnosis 420) were autopsied at the Department of Forensic Medicine Karolinska Institutet, Stockholm Of the ASHD deaths at least 99 had occurred in the Stockholm region Information was gathered from autopsy protocols and police re ords the latter containing details such as time and place of death activity at onset and prior symptomatology and medical care *These facts* we e obtained from testimonies and police investigations made routinely in every case

The term sudden death was used when the follow ing cr teria we e met death during mental or physi al act vity other than rest, where t me had not allowed the victim to be brought under medical care Sudden death at rest was found diff cult to evaluate and consequently this category of deaths was excluded from the study

Moderate physical or mental acti ity" included activi ties l ke walking, household work and reading, etc

Heavy phys cal exercise" w s used to describe activi ties such as running walking upsta rs and such l ke

The term mental stress was appl ed to circumstances such as quarrel gambling, etc

No prev ous symptoms of ASHD" were cons dered to ha e been present in cases where it had been stated that the patient had not been under medical care for heart disease and no ment on of symptoms suggestive of ASHD prior to death had been made

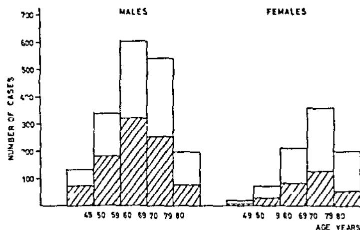


Fig 1 Age and sex distribution of deaths from ASHD outside the hospitals ////// sudden deaths ______ not sudden deaths

RESULTS

According to the above mentioned criteria sudden death occurred in 1213 cases (912 males and 301 females) out of the total of 2678 deaths (1819 males and 859 females) The overall male female ratio was 2 1 1 as compared to 3 1 for the sudden deaths

The mean age in the whole series was 65 7 years for males and for females 71 2 years a difference of 5 5 years In the group of sudden deaths the age difference was 4 4 years the mean age of the males and females being 65 0 and 69 4 years respectively Age and sex distributions of the deaths are shown in Fig 1

Activity preceding sudden death is given in Table I Following the above definition deaths at rest were excluded from the sudden deaths It is seen that among males heavy physical exercise and mental stress together preceded 26% of the deaths below the age of 50 and close to 16% in the higher age groups However in the female sudden deaths these activities were noticed only in a negligible minority

From Fig 2 it is seen that in all age groups the male sudden deaths occurred nearly as frequently outside home (work included) as at home A somewhat smaller proportion of the females died outside their homes

As seen in Fig 3 recent myocardial infarction was diagnosed at autopsy in 34% of male sudden deaths below the age of 50 The corresponding female age group was too small to permit comparison From the age of 50 and on recent myocardial infarction was found in approximately 20% in both sexes

A myocardial infarction more than one week old was found in 32% of the males below the age of 50 The corresponding figure for the higher

Table I *Activity preceding sudden witnessed death (death at rest excluded according to definition)*

Age	Moderate physical or mental activity	Heavy physical exercise	Mental stress	Total
Males				
–49	54 (74%)	18 (25)	1	73 (100)
50–59	154 (84%)	25 (14)	5 (?)	184 (100)
60–69	267 (83%)	45 (14)	11 (3)	3_3 (100)
70–	290 (88%)	31 (9%)	11 (3%)	332 (100%)
Females				
–49	8	0	0	8
50–59	_9	0	0	_9
60–69	81	2	1	84
70–	175	2	3	180

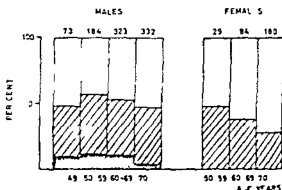


Fig 2 Place of sudden deaths Females –49 years not included (n 8) ______ death at work //// death outside home (except work) death at home

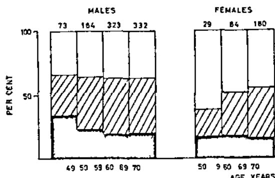


Fig 3 Autosopy findings in sudden deaths ascribed to ASHD Females −49 years not included ($n = 8$) [box] recent myocardial infarction (≤ one week old) ////// myocardial infarction > one week old [box] ASHD without reference to thrombotic occlusion

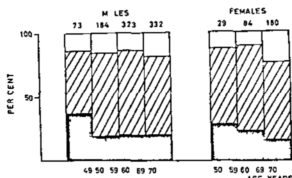


Fig 4 Sudden death and previous symptoms suggestive of ASHD Females −49 years not included ($n = 8$) [box] no previous symptoms suggestive of ASHD ////// previous symptoms of ASHD [box] uncertain

age groups was 43 % with a narrow range In the females below the age of 60 myocardial infarction more than one week old was seen in 21 % The older females were found to have an old myocardial infarction nearly as frequently as the males of corresponding age (Fig 3)

The average proportion of sudden deaths from ASHD without reference to thrombotic occlusion was found to be somewhat higher in females than in males (Fig 3)

The distribution of myocardial ruptures is shown in Table II The overall proportion of sudden deaths due to myocardial rupture in males and females was 3 2 and 4 7 % respectively An increased rate of myocardial ruptures with age was seen in both sexes In all cases of myocardial rupture a recent myocardial infarction was diagnosed and hence classified as recent myocardial infarction in Fig 3

Previous symptoms of ASHD were considered absent in 249 (191 males and 58 females) out of 1213 sudden deaths (20) A presentation of these deaths is given in Fig 4 Sudden death as first manifestation of ASHD was recorded in 37 % of the males below the age of 50 In the older males the corresponding figure was close to 20 %

Although there was only a small number of women in the younger age groups a similar tendency towards a higher percentage of sudden death as first manifestation of ASHD was found in the females

Autopsy findings in subjects without previous symptoms of ASHD are shown in Fig 5 Recent myocardial infarction was found in 63 % of the males below the age of 50 Beyond this age a finding of recent myocardial infarction was made in about 25 % of both sexes A myocardial infarction more than one week old was found in approximately 20 % of the males unrelated to age ASHD without thrombotic occlusion was found in 19 of the males below the age of 50 and

Table II *Myocardial ruptures in sudden deaths*

Age −49	50–59	60–69	70–	Total
Males				
0/54	/184 (1)	12/323 (4)	15/33 (5)	29/91? (3 2)
Females				
0 8	0 29	2/84 ( )	1 /180 (7)	14/301 (4 7)

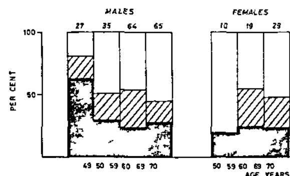


Fig 5 Autopsy findings in *sudden deaths without previous symptoms* suggestive of ASHD [box] recent myocardial infarction (≤ one week old) ////// myocardial infarction > one week old [box] ASHD without reference to thrombotic occlusion

in the higher age groups this was seen in approximately 50%. In the females the autopsy finding were essentially the same

DISCUSSION

Like other studies on deaths from ASHD outside the hospitals based on the files of Medical Examiners and Coroners the present one represents a selected material An unknown number of such deaths in the Stockholm region was not referred to the Department of Forensic Medicine In the Stockholm region the Department of Forensic Medicine in addition to carrying out strictly forensic investigations also has a function similar to that of the Coroner or Medical Examiner performing autopsies in cases of non violent death when a doctor sufficiently familiar with the patient to be able to issue a death certificate is not available Of all cases brought to the Department during the time studied autopsy was carried out in 85% Autopsy was not carried out in cases when a physician was willing to issue a death certificate provided that the police had no objection to this procedure It was thought that the figures given in the present study could be regarded as an estimated minimum of sudden deaths from ASHD outside the hospitals in the Stockholm region a city community of about one million inhabitants during the years studied

The above mentioned criteria of suddenness were set arbitrarily No uniform definition has as yet appeared in other studies as has been pointed out by Kuller (6) in his extensive review of the literature on this subject

The largest number of sudden deaths was recorded in the higher age groups in both sexes the mean age of the males and females was 65 0 and 69 4 years respectively The average age of death from ASHD at a general hospital in Stockholm like the Serafimer hospital during the period studied was 68 0 (males) and 72 0 years (females) a slight difference to which no significance can be attributed owing to the above mentioned limitations of the study However the percentage of sudden deaths according to the criteria used in the present study was slightly higher in the lower age groups of both sexes (Fig 1) According to the definition of sudden death almost all cases of sudden death were witnessed the lower percentage of sudden deaths in the old females could thus be a consequence of a higher proportion of women living alone due to the longer life span of the females

In the literature there is little information about circumstances associated with sudden deaths In the present study detailed police records made reconstructions possible It may seem remarkable that so few deaths occurred in association with physical exercise and mental stress Further confirmation on this point is needed

On account of the low coincidence of heavy physical exercise and mental stress with sudden death it was not surprising to find so few deaths occurring at work Because of possible medicolegal implications it is assumed that a relatively higher proportion of the deaths at work was referred to the Department of Forensic Medicine and that autopsy was carried out in every case This in turn could lead to an overrepresentation of sudden deaths occurring at work Assuming that 40 hours per week were spent at work and that vacation and public holidays together accounted for one month per year the time spent at work would represent about 20% of the year In the present study no more than 12% of the male sudden deaths below the age of 70 occurred at work Kuller et al (7) in an epidemiological study on sudden deaths between 40 and 64 years found that 15% of the deaths occurred at work where it was estimated that 25 to 30% of the time was spent

Postmortem findings should be considered in relation to the sudden character of death Myocardial infarction cannot be demonstrated by routine technique as used in this study until several hours after its emergence (2) However examining serial sections and the microscopic appearance Baroldi (1) could demonstrate no acute or recent coronary occlusion in 54 % of 125 sudden but not unexpected "coronary heart deaths A normal myocardium was found in 31% of these cases Coeval myocardial and coronary lesions were demonstrated in only 19% of the 125 cases In a very detailed study including post mortem angiography and microscopic dissection Crawford (3) found recent thrombotic occlusion in 41% of 100 sudden deaths ascribed to ASHD The results of these studies may serve as an explanation for the relatively frequent finding of ASHD without reference to thrombotic coronary occlusion in the present study

Myocardial rupture as a cause of sudden death was found to be increasingly frequent with age This finding is supported by two British studies (4 8) Mitchell and Parish (8) in a study of 36 cases of myocardial rupture found that half of these occurred within 24 hours after the onset of symptoms The same authors found a female preponderance the male female ratio being 1 1 2 Crawford and Morris (4) reported a male female ratio of 1 0 6 under the age of 70 Above this age the ratio was reversed to 1 1 7 Both studies were based on hospitalized and Coroner cases combined In two Swedish studies on hospitalized patients Sievers et al (9) found a male female ratio of myocardial ruptures of 1 1 2 In a later study Sievers (10) reported a male female ratio of 1 1 4 Agreeing with these results the male female ratio in the present study was found to be 1 1 5

It is rather interesting that in males above the age of 50 the proportion of cases in which sudden death was the initial manifestation of ASHD was close to 20° of all sudden deaths from ASHD In the youngest male group sudden death as first manifestation of ASHD was found in 37° of the sudden deaths a not unexpected difference Kannel et al (5) in a report from the Framingham study found that sudden death was the first manifestation of ASHD in 20 of all new cases of ASHD exclusive of angina pectoris The results of this study indicate that even in a community with a carefully planned search for signs of ASHD a substantial proportion of sudden deaths occurs before any clinical signs of ASHD have appeared

Recent myocardial infarction might be expected to be a more common finding in sudden death not preceded by any symptoms of ASHD because of a more careful search for the cause of death Baroldi (1) found a normal myocardium as judged from serial sections and microscopic examination in 53% of 116 sudden coronary deaths without previous symptoms of ASHD A recent thrombotic occlusion has likewise been found in 53% However coeval thrombotic occlusion and myocardial lesion were found in only 22 It is rather puzzling that such a high proportion of fatal events seems to lack anatomical evidence

The total annual numbers of sudden deaths from ASHD outside the Stockholm hospitals during the time studied were not possible to record However the rough figure of 300 cases recorded in the present study during each of the last three years (1964 1965 and 1966) could be regarded as a minimum number of sudden deaths from ASHD outside the hospitals in the Stockholm region a city community of about one million inhabitants In the light of the present techniques of resuscitation and management of serious arrhythmias this largely overlooked category of patients now dying medically unattended stands out as one of the most urgent problems of coronary heart disease to-day

REFERENCES

1 Baroldi G Amer J Cardiol 16 859 1965
2 Bjurulf P., Garlind T & Sternby N H Acta med scand Suppl 474 1967
3 Crawford T Evolution of the atherosclerotic plaque (ed Richard J Jones) p .79 University of Chicago Press Chicago 1963
4 Crawford M D & Morris J H Brit med J 2 16 4 1960
5 Kannel W B Kagan A Dawber T R & Revotskie H Geriatrics 17 675 1966
6 Kuller L J chron Dis 19 1165 1966
7 Kuller L Lilienfeld A & Fisher R Medicine 46 4 1967
8 Mitchell J R & Parish D J Brit med J . 16 6 1960
9 Sievers J Blomquist G & Biorck G Acta med scand 169 95 1961
10 Sievers J Acta med scand Suppl 175 1963

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IN VITRO MIGRATION OF PERIPHERAL HUMAN LEUCOCYTES IN CELLULAR HYPERSENSITIVITY

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Abstract The influence of various antigen concentrations upon the in vitro migration of peripheral leucocytes from persons with cellular hypersensitivity to *B abortus* Bang is investigated An inhibition as well as a stimulation of the cell migration can be demonstrated The dual effect of the antigen upon the migration cultures seems to be dependent upon two factors first the antigen concentration, and second the sensitivity of the cell donor High antigen concentrations lead in all cases to inhibition of the cell migration Low antigen concentrations tend to result in a stimulation of the cell migration if the cells are of a low to moderate degree of sensitivity while cells of high sensitivity are still inhibited at the low concentrations

According to the generally accepted view the action of antigen in vitro upon immunocompetent cells results in an inhibition of the cell migration if the cells originate from an organism in a state of cellular hypersensitivity to the same antigen This observation has been confirmed in many animal experiments (2 3 4 6 9 10 12 13 14) and recently in studies in man (18 19) The inhibition of the cell migration corresponds well to the sensitivity of the cell donor as expressed by the delayed intracutaneous reaction in a way that a more pronounced inhibition of the migration is paralleled by a more intense intracutaneous reaction There have however been a few reports indicating that under special experimental circumstances a stimulation of the cell migration may be seen as well (7 11 15 16)

Juhasz Schaffer using fragments from the spleen and the kidney was able to show a stimulation of the cell migration in the presence of tuberculin in rather low concentrations if the cells originated from tuberculin sensitized animals Hall and Scherago in experiments with peripheral human leucocytes showed that tuberculin sometimes caused an inhibition sometimes a stimulation and finally in some cases no alteration of the cell migration could be observed The authors considered the results contradictory and concluded that the method in their hands was not able to demonstrate cellular hypersensitivity in vitro

Švejcar and Johanovsky (15) in their study with spleen fragments demonstrated that cells of tuberculin sensitive guinea pigs in the presence of specific antigen showed an increased migration activity in the first hours of incubation compared with the control cultures Later in the experiments the usual inhibitory effect of tuberculin was observed so that the final result after 24 hours was an inhibition of the cell migration

Švejcar and coworkers (16) were also able to demonstrate that in some cases the cells were permanently stimulated if low antigen concentrations were applied

It thus seems suggestive that there is a relationship between the antigen induced inhibition and stimulation of the migration of immunocompetent cells in vitro and that both types of reaction may be expressions of cellular hypersensitivity

The purpose of the present work is to study further this interrelationship and to analyze some of the factors involved in the action of the antigen upon the cell migration Brucella hypersensitivity is used as an experimental model because the antigen induced inhibition of migration of peripheral human leucocytes in vitro has proved to be a specific parameter of cellular hypersensitivity (18)

MATERIAL AND METHODS

The material consisted of 97 brucella positive and 82 brucella negative persons

Table I *The migration indices of brucella negative and brucella positive persons at various antigen concentrations*

N = number of observations $\bar{X}$ = mean value S = standard deviation P = statistical difference of the mean value in relation to the brucella negative observations.

Antigen conc (bact/ml)	Brucella neg. 0 78 1 11 N	$\bar{X}$	S	Brucella pos. 0 71–0 76 N	$\bar{X}$	S	P	0 61–0 70 N	$\bar{X}$	S	P	0 51–0 60 N	$\bar{X}$	S	P	≤0 50 N	$\bar{X}$	S	P
50 mill	82	0 90	0 07	74	0 73	0 02	<0 001	39	0 65	0 03	<0 001	48	0 55	0 03	<0 001	16	0 4[illegible]	0 09	<0 001
25 mill	33	0 93	0 08	8	0 84	0 10	<0 05	0	0 74	0 [illegible]1	<0 01	20	0 68	0 07	<0 001	8	0 58	0 07	<0 001
10 mill	53	0 96	0 08	19	1 11	0 17	<0 01	35	0 91	0 15	>0 05	31	0 77	0 10	<0 001	10	0 65	0 11	<0 001
5 mill	20	0 99	0 08	10	1 15	0 17	<0 02	19	1 02	0 17	>0 05	13	0 80	0 11	<0 001	—	—	—	—
2.5 mill	22	0 98	0 06	7	1 23	0 13	<0 01	6	1 36	0 25	<0 02	—	—	—	—	—	—	—	—
1 mill	21	1 01	0 11	11	1 08	0 21	>0 05	16	1 21	0 27	<0 01	20	0 99	0 28	>0 05	10	0 73	0 16	<0 01

Brucella hypersensitivity was defined in the following way

A *migration index below 0 78 at an antigen concentration* of 50 mill brucella bact per ml (see below) which has been shown to be the borderline between the brucella positive and brucella negative observations (18)

Persons were considered brucella negative if they did not fulfil the above mentioned criterion

The hypersensitive group consisted of 37 spontaneous *brucella positive persons and of 65 persons examined after* the injection of 1000 mill killed brucella bact (*Brucella abortus* Bang) Some of these cases were studied repeatedly after the vaccination

Leucocyte migration studies

Migration studies were performed with peripheral human blood leucocytes according to the technique initiated by Bendixen and described in detail in a previous paper (17)

The action of the antigen upon the cell migration was expressed in the migration index M /M where M represents the average migration area of the antigen containing cultures and M the average migration area of the control cultures Thus the numerical value of the migration index expresses whether the migration has been stimulated or inhibited by the antigen An index exceeding 1 00 indicates stimulation and an index less than 1 00 inhibition of the cell migration

In each experiment the migration index was determined at a high concentration of brucella antigen, i e 50 mill bact per ml Furthermore the migration indices were determined at one or more of the following concentrations of brucella antigen 25 mill., 10 mill 5 mill., 2.5 mill and 1 mill bact per ml The cells in each experiment were examined at at least two different antigen concentrations Because of the rather limited amount of cultures which could be obtained it was not possible in each experiment to determine the migration indices at all the different antigen levels

RESULTS

Table I shows the distribution of the migration indices at the different antigen concentrations The brucella positive observations have been divided into four groups according to their degree of sensitivity i e the migration indices at an antigen concentration of 50 mill bact per ml The least sensitive group consists of all observations with migration indices from 0 71–0 76 the next two groups comprise values from 0 61–0 70 and 0 51–0 60 and the most sensitive group represents migration indices below 0 50 This separation is of course to some extent arbitrary but builds upon the fact that the migration indices at 50 mill bact per ml have been shown to be well correlated to the sensitivity of the cell donor as expressed by the intracutaneous reaction (18)

It appears (Table I) that at the low antigen concentrations there is a tendency to stimulation of the migration as indicated by migration indices over 1 00

On the other hand the migration indices from the brucella negative group do not show this alteration The values are all placed around 1 00 with minor variations at the different antigen concentrations

The mean values of migration indices in brucella positive cultures at the different antigen levels are significantly different from the mean values of migration indices in brucella negative cultures at nearly all levels with a few exceptions In these cases however the standard deviations are significantly different

In order more clearly to illustrate the relationship between the concentration of antigen and its influence upon the cell migration the mean values have been plotted in a diagram (Fig 1) At the high antigen concentration the migration indices from the brucella positive persons are all well

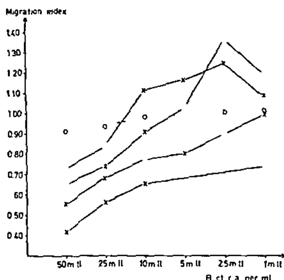


Fig 1 The migration indices at various antigen concentrations x—x brucella positive O O brucella negative

below 1 00 With decreasing antigen concentrations in the least sensitive groups first a disappearance of the migration inhibition is seen followed by a stimulation whereas the migration of the highly sensitive group remains inhibited at the lowest concentrations although to a smaller degree The curve thus seems to be biphasic starting with low indices followed by an interval with indices within the normal range continuing with high indices and finally going down into the normal range It also appears from the diagram that at a fixed low antigen concentration a wide distribution of indices is observed indicating that in some cases the migration has been stimulated in some inhibited and in some left uninfluenced by the antigen

DISCUSSION

The data presented indicate that the action of antigen upon sensitized cells may influence the migration in apparently two ways inhibition or stimulation The final result seems to be dependent upon at least two factors 1 the antigen concentration and 2 the sensitivity of the cells At a fixed sensitivity the outcome will depend upon the antigen concentration in such a way that high concentrations induce an inhibition and low concentrations a stimulation If on the other hand the antigen concentration is fixed at a relatively low level the outcome will depend upon the sensitivity of the cells a highly sensitive culture being inhibited and a less sensitive culture stimulated or perhaps apparently uninfluenced

Inhibition and stimulation may thus both be considered expressions of cellular hypersensitivity in vitro and seem to be closely interdependent

The above mentioned experiments by Juhasz Schaffer (11) Hall and Scherago (7) and Švejcar and Johanovsky (16) may support this theory Juhasz Schaffer using spleen and kidney fragments from guinea pigs was able to show only stimulation after addition of tuberculin antigen The antigen concentration was however low namely a 1 3000 dilution of O T whereas in a similar system where inhibition was a constantly occurring phenomenon the concentration was 1 100 of O T (14) The two experiments cannot be directly compared first of all because the degree of sensitivity of guinea pigs must be different due to the various immunization procedures applied Nevertheless it seems reasonable to conclude that the stimulation seen in Juhasz Schaffer s experiments at the low antigen concentration is related to the inhibition seen in Švejcar and Johanovsky s experiments at the high antigen concentration in the same way as described in the brucella system Švejcar and Johanovsky in their later experiments (16) have more directly established this proposed connection They investigated the migration of sensitized spleen cells from guinea pigs at various antigen concentrations and found that at the lower concentrations there was a tendency to stimulation of migration while the migration was always inhibited at the high concentrations The stimulation observed was statistically significant at the 5% level at two of the low concentrations but otherwise the picture was a wide distribution of indices at the various antigen levels This might be expected because no attempt was made to divide the material according to the sensitivity of the cell donors In other words only one of the factors which determine the final outcome of the cell migration was analyzed namely the antigen concentration

Conversely Hall and Scherago in their experiments with human leucocytes tried to separate the patients somewhat according to the degree of sensitivity i e the severity of the tuberculous infection The experiments were as a whole not

very successful because the authors were not able to show a constantly occurring tuberculin induced inhibition of migration not even in the most sensitive group This negative result may be explained by the fact that the antigen concentration applied was too low Indices from the patients belonging to the least hypersensitive groups in some cases showed values exceeding 1 00 indicating a stimulation of the migration which was never observed in the highly sensitive groups The deviations were not statistically significant and the main impression was again a wide distribution of indices. These experiments probably represent an analysis of the other factor involved namely the sensitivity of the cell donor

In the present experiments the antigen concentration as well as the sensitivity of the cell donor has been considered and this double aspect seems to give a clearer picture of the relationship between stimulation and inhibition of the cell migration. Apart from the theoretical importance of this relationship some facts of practical value in the detection of cellular hypersensitivity in vitro can be extracted In the first place a stimulation of migration may be considered an expression of cellular hypersensitivity just as specific as an inhibition of migration. Secondly it is necessary to use very high, i.e subtoxic antigen concentrations to ensure an inhibition of migration in all cases examined. If too low antigen concentrations are used the picture will be confusing with stimulation, inhibition or no changes of the migration at all, and it will be impossible to correlate the findings with the intracutaneous reaction. It should also be clear that the degree of stimulation determined at a single antigen concentration cannot be used as a parameter of cellular hypersensitivity because it is impossible to predict at which point on the biphasic curve the value is recorded in other words whether it is placed on the ascending part, the top or the descending part.

If the experimental circumstances do not allow the application of an antigen concentration as high as desirable there still exists the possibility to determine the sensitivity of the cell donor by using falling concentrations of antigen. Determination of migration indices at various antigen concentrations will permit at least part of the biphasic curve to be established thereby providing a more exact expression of the degree of sensitivity than a determination of the migration index at a single antigen concentration.

The mechanism of the stimulation of migration is just as obscure as the mechanism of inhibition, but it seems reasonable to assume that both are due to the same fundamental biologic process In animal experiments it has been shown that the inhibition is dependent upon a substance excreted by the sensitive lymphocytes upon contact with the antigen (1 5 8) Preliminary experiments have shown that a similar factor may be instrumental in the in vitro system used in the present study

The effect upon the migration might simply be related to the resulting concentration of active substance in the tissue culture medium a high concentration resulting in inhibition and a low concentration in stimulation Investigations to prove this hypothesis are in progress The experiments by Švejcar and Johanovský (15) indirectly support this theory As earlier mentioned, they determined the migration indices at different hours after the addition of antigen and observed that in the beginning there was a stimulation which later disappeared and was gradually converted into an inhibition of the cell migration The most likely explanation seems to be that the concentration of the active substance is at first low resulting in stimulation and later as the production continues and the concentration rises the cells will be inhibited in their migration

The interaction between sensitivity and antigen concentration as visualized by the biphasic curve thus seems to be an expression of a general biologic phenomenon in which small stimuli result in stimulation or proliferation while an increase of the stimuli leads to inhibition or destruction.

ACKNOWLEDGEMENTS

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REFERENCES

1 Bloom, B R. & Bennett, B Science 153 80 1966
2 Carpenter R. R. J Immunol 91 803 1963
3 Carpenter R. R. & Brandriss, M W J exp Med. 120 1231 1964
4 David, J R., Al Askari, S., Lawrence H S & Thomas, L. J Immunol. 93 264 1964

5 David J R. Proc nat. Acad Sci. 56 7_ 1966
6 George M & Vaughan J Proc Soc exp Biol (N Y) 111 514 196
7 Hall, H E & Scherago M Amer Rev Tuberc 75 807 1957
8 Halpern, B Storb U & Fray A. Nature (London) 215 400 1967
9 Heilman D H Howard D H & Carpenter C M J exp Med 107 319 1958
10 Johnson R. W & Scherago M Amer Rev resp Dis 81 96 1960
11 Juhasz Schaffer A. Z. Immun Forsch. 56 25 19_8
12. Morn J K. J exp Med 64 355 1936
13 Rich A R. & Lewis M R. Bull Johns Hopk Hosp 50 115 193_
14 Švejcar J & Johanovský J Z. Immun. Forsch. 1_2 398 1961
15 — Z. Immun. Forsch 1 8 1 1965
16 — Z Immun Forsch 131 301 1966
17 Søborg. M & Bendixen G Acta med scand 181 247 1967
18 Søborg M Acta med scand 18_ 167 1967
19 Thor D E Science 157 1567 1967

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PERCUTANEOUS RENAL BIOPSY ON URAEMIC PATIENTS AIDED BY SELECTIVE ARTERIAL ANGIOGRAPHY AND ROENTGEN TELEVISION

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Abst act Selective renal arterial angiography and television monitored fluoroscopy have been used in locating the kidney at percutaneous renal biopsy in 68 patients including 40 with uraemia. This procedure makes the renal arteries and the contour of the kidney clearly visible The tip of the biopsy needle can be placed at a su table point on the kidney surface away from the arteries and the risk of bleeding seems thus to be reduced No serious clinical complications occurred So far no toxic damage to the renal tissue has been noticed referable to the contrast medium Renal angiography is preferable as a means of localizing the kidney when needle biopsy is to be performed in cases of chronic renal failure in obese and heavily bu lt patients and in cases of nephrosis and overhydration In these cases the method merits rout ne use It is not necessary to p rform renal arterial angiography as an aid to renal biopsy in patients with normal or moderately impaired renal function if the k dneys can be visualized at fluoroscopy directly or after urography In acute renal failure we think that retrograde pyelography can be used but it is not the preferable method

Television monitored fluoroscopy has proved to be a useful aid in the procedure of percutaneous renal biopsy (3 4 6) A prerequisite is that the contour of the kidney is sufficiently clearly definable on the television screen

One can only occasionally see the kidneys directly at fluoroscopy In patients with normal renal function intravenous administration of a contrast medium which accumulates in the renal parenchyma (urography) will markedly increase the visibility of the kidneys Large kidneys of severely uraemic patients (creatinine exceeding 6 mg per 100 ml of serum) are occasionally displayed directly if the patients are effectively dehydrated and adequately purged and if intestinal gas is eliminated (6) In these cases however urography will never improve the visibility of the kidneys The contours of small kidneys are according to our experience never visible at television monitored fluoroscopy if the patient has advanced uraemia even if an intravenously administered contrast medium is given in large and repeated doses and for several (up to 24) hours before the biopsy

We have performed renal biopsy on uraemic patients after the renal pelvis has been filled with a contrast medium by retrograde pyelography on the side where the biopsy was done (7) In that paper we have reported attempts at renal biopsy in connection with renal arterial angiography Experience has now been gained concerning the value of this procedure

Selective renal arterial angiography has been performed bilaterally on all the patients as part of the investigation of their disease and to exclude malformations or morphological changes that would make renal biopsy dangerous to the patient (arterial aneurysms unilateral aplasia or advanced unilateral hypoplasia of the kidney) The needle biopsy was usually done immediately after the angiography In a few cases angiography and biopsy could not be combined A catheter was then inserted into the renal artery only to allow renal biopsy to be carried out

TECHNIQUE

The patients are purged with castor oil on the day before the b opsy and should not be gi en any fluid for the last 1. hours A water enema (1 r 1) s given two hours before the biopsy is to be done One and a half hours before the b opsy water-soluble vasopressin (Postacton®) is injected subcutaneously No mal we ght



Fig 1 Roentgen film exposure taken during inje t on of contrast medium into the renal artery showing the biopsy instrument introduced into the right kidnev of a patient weighing 63 kg. His creatinine was 8 7 mg per 100 ml of serum The kidney measured 10 5 × 5 4 cm and the renal cortex 3–4 mm in thickness on the roentgen film The biopsy yielded 11 mm of renal tissue which showed chronic glomerulonephritis on microscopical examination No complications were noted

patients receive 10 IU heavy patients (more than 80 kg) 15 IU and light patients (less than 55 kg) and children 5 IU The patients are premedicated with morphine scopolamine according to weight and ag

The position of the first and second lumbar vertebrae and the tip of the twelfth rib are marked with a lead shot, which is fastened with adhesive plaster on the skin of the back of the patient. After local anaesthesia a red Ödman Ledin catheter (AB Kifa, Stockholm) is inserted by the Seldineer technique into the femoral artery through the groin The procedure is controlled by fluoroscopy using an image intensifier combined with television. Selective renal diagnostic angiography is then performed bilaterally (1 8) after which the catheter is left with its tip lying in the abdominal aorta The roentgen films are examined and it is decided on which side the biopsy is to be done The bent tip of the catheter is placed in the renal artery on the side where the biopsy is planned and the patient is then carefully turned over into the prone position He should lie with his legs stretched and not co-operate in order to lessen the risk of the tip of the catheter slipping out of position After the patient has been turned, a check is made by fluoroscopy to make sure that the tip of the catheter is still in the renal artery

When 1–2 ml of the contrast medium (60 Urografin®) is injected into th renal artery catheter the operator can watch on the television screen the contrast medium passing rapidly through the large arteries and accumulating in the parenchyma rendering the kidney visible for a minute or two The track down to the surface of the kidney is infiltrated with a local anaesthetic and by measuring the length of the free end of the needle one can calculate how deeply the tip of the needle is situated The position of the tip of the anaesthetic needle on the renal contour is checked by fluoroscopy and changed if necessary (7) A Franklin Vim Silverman needle 12 cm long and sharply ground is then introduced the same way and to the same depth as the anaesthetic needle The resistance offered by the renal parenchyma and the movements of the instrument with the respiration and arterial pulse wave of the patient indicate that the kidney has been reached Contrast medium is injected into the renal artery catheter and the operator checks by fluoroscopy that the tip of the biopsy instrument is situated in the lower pole of the kidney at a safe distance from the large arteries (Fig 1) The patient is told to hold his breath and the biopsy is done with vigorous rapid movements If no renal tissue or too short a piece is extracted at the first attempt the biopsy should be repeated at most twice and the position of the needle should be checked before each attempt Th biopsy tissue is arranged in spiral form on a dry slide and fluid agar agar is poured over it By examination under the dissection microscope it can be ascertained that th tissue contains glomeruli (5)

The patient is confined to bed for observation until the following day

RESULTS

Renal biopsy by the technique described has been performed on 68 patients Thirty four of these had severe uraemia (creatinine 6–15 mg per 100 ml of serum) and sixteen slight uraemia Renal function was normal or slightly impaired in 18 patients The pieces of tissue retrieved at biopsy were sufficient for diagnosis in 57 cases We have found the length and sharpness of the biopsy instrument to be of great importance for the outcome of the biopsy attempts (7)

Complications referable to the arterial punctures were no more frequent in our cases than in those described by others (1) We could not find that any complications were caused by the tip of the catheter being left in the renal artery for a relatively long time The amounts of contrast medium given during fluoroscopy in connection with the biopsy procedure (totally 3–12 ml) are the same as those administered during diagnostic selective renal angiography The repeated injections of contrast medium do not seem to give rise to any clinically demonstrable disadvantages The uraemic state of the patients was unchanged af

ter the biopsy or increased at the same rate as before the procedure Our results accord with those of other investigators who have found that an impairment of the renal function cannot be demonstrated in healthy persons after renal angiography (2)

Histological examination under the light microscope has so far not shown any evidence of acute toxic effects in the retrieved pieces of tissue In the biopsy specimens from the patients with uraemia the histopathological changes caused by the disease were so advanced that it could not be decided whether further acute damage had been caused In the material there are three patients with proteinuria but no other evidence of renal disease The biopsy specimens showed histologically normal renal tissue in these cases

No serious clinical complications were seen apart from transient shock immediately after the biopsy in one patient with amyloidosis There were no signs of local bleeding It is not clear whether the shock was due to blood loss pain or hypersensitivity to the contrast medium The patient had reacted in the same way at a previous examination when he was given a contrast medium intravenously One patient had gross haematuria for three and another intermittent haematuria for 14 days

We have not performed renal arterial angiography after the biopsies and the material has not been investigated with regard to late complications The possibility of intrarenal damage caused by the biopsy attempts e g arteriovenous fistulas cannot be excluded So far however we have not noticed any clinical symptoms indicative of late complications

COMMENTS

The method described enables the clinician to carry out renal biopsy in uraemic patients even if their kidneys are small and renal function is greatly impaired It allows repeated and careful checking of the position of the biopsy needle in relation to the contour of the kidney and the large arteries

The methods of displaying the kidneys at renal biopsy should be chosen with regard to the body build of the patient the nature of the renal disease and the degree of impairment of renal function

In patients with normal renal function the kidneys can as a rule be seen directly at fluoroscopy after administration of a contrast medium intravenously If the patient is oedematous very fat or heavily built the organ is often not clearly visible even if urography is performed Then renal biopsy should be done in connection with renal angiography

In cases of moderate uraemia (creatinine less than 6 mg per 100 ml of serum) repeated injections of contrast medium intravenously and injections of vasopressin subcutaneously will sometimes suffice to render the kidneys visible on the television screen If necessary arterial angiography is performed to display the kidneys in these patients

Patients suffering from chronic renal failure and advanced uraemia often have arterial hypertension and a considerable bleeding tendency At arterial renal angiography the vessels as well as the renal parenchyma are displayed This augments the possibility of performing the biopsy in an area free from large arteries although the kidneys often are shrunken and the renal cortex considerably reduced in thickness

Two of us (Lindqvist and Nystrom) have used retrograde pyelography as a localizing aid when performing percutaneous renal biopsy in seven patients with advanced uraemia whose kidneys could not be seen directly at fluoroscopy (7) Because the contrast medium remains in the renal pelvis for a considerable time the operator is given ample time to watch the biopsy needle move together with the renal pelvis as the patient respirates There is also time to place the tip of the needle in a position immediately beneath the renal capsule ensuring the best chance of obtaining an adequate specimen Anyhow as the method always carries a risk of introducing bacteria into the renal pelvis and any acute infection of the kidney in a patient with advanced uraemia will have serious consequences we have now abandoned retrograde pyelography as a means of localizing the kidney at renal biopsy and prefer to use selective arterial angiography instead However we think that retrograde pyelography can still be used to aid in a biopsy attempt if a catheter must be passed into the renal pelvis for diagnostic reasons e g to exclude any postrenal obstacle in a patient with acute renal failure and anuria and where selective renal angiography is

not planned. Also when lacking facilities at renal angiography or when the procedure is contraindicated (hypersensitivity to the contrast medium) retrograde pyelography merits consideration as a method to visualize the kidney when renal biopsy is to be carried out

REFERENCES

1 Boijsen, E Acta radiol (Stockh) Suppl. 183 1959
2. Bower J D., Mayer J H & Lester R G Surgery (St. Louis) 60 545 1966
3 Buenger R. & Kark, R. Lancet 1 904 1966
4 Edholm, P., Fernstrom I., Lindblom, K. & Seldinger S I Acta radiol. (Stockh.) Suppl. 216 1962.
5 Larsson, O Lindqvist, B & Nystrom, K. Nord Med 74 845 1965
6 Lindqvist, B Acta med scand 181 97 1967
7 Lindqvist, B & Nystrom, K. Scand J Urol Nephrol 1 297 1967
8 Seldinger S I Acta radiol. (Stockh.) 39 368 1953

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THE OBSERVER VARIATION IN THE MEASUREMENT OF ARTERIAL BLOOD PRESSURE

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Abstract Observer errors and observer variation have been studied in a survey of blood pressure covering about 70 000 subjects in Bergen Norway carried out by 19 specially trained nurses under cond tions standardized as much as possible Substantial differences were found between extreme observers concerning the mean values of the read ngs in age and sex groups while the majority only showed insignificant differences Read ng characteristics were supposed to be the reason for individual observers keeping the same ranking position vis à vis the others from age group to age group in both sexes A number of observers had however a different ranking in systolic from that in diastolic readings Terminal digit preference in the readings was found only to a small degree and only in a few of the observers The special apparatus of the London School of Hygiene was found to reduce but not to eliminate this error When the results obtained with the latter apparatus were compared to standard apparatus results no differences in the reading characteristics were found related to the sex, age and blood pressure level of the subjects examined

Conclusion observer variation in blood pressure examination may be substantial and may affect the me values found in population surveys even if the observ are carefully selected and thoroughly trained

The measurement of arterial blood pressure is a valuable method of assessing the condition of the heart and circulatory system in individuals and groups Because of its ease of application it has been used extensively in epidemiological studies which have contributed greatly to the knowledge of the cardio-vascular conditions in population groups all over the world

The evaluation of the blood pressure findings has however been complicated by substantial experimental error (5) Clearly this error has to be kept in mind in the practical use of blood pressure readings and in the planning of epidemiological studies

The Bergen Blood Pressure Committee is constituted of members from the University of Bergen, School of Medicine Medical Departments A and B and Bergen Helseråd.

Present knowledge indicates (6 8) that the true blood pressure undergoes variation depending upon factors such as sleep physical activity emotional status and environmental influences In addition other factors influence the indirect measurement of the pressure details of the instrument used such as the length and width of the cuff the rate of inflation and deflation of the cuff the position of the arm measured are important (5 7 9) Technical details of the sphygmomanometer such as the reading column may result in some readings being too high and others too low and in terminal digit preference (7) The technical competence of the person performing the examination will also influence the results Mental concentration reaction time hearing acuity interpretation of sounds visual details and the separation of auditory and visual cues in the observation of a moving mercury column may all be assumed to be of importance (3 7) The relationship between observer and subject may be relevant and different results according to age sex and other characteristics of the observer may be obtained Such factors may substantially influence the blood pressure readings in individuals and may also distort the frequency distribution of group values and affect the mean values (7) Consequently the errors made should be known and if possible avoided or corrected for This will be of special importance in population studies where the blood pressure is measured by several observers Here the variation between observers should be recognized

MATERIAL AND METHODS

A blood pressure survey was carried out in the population of Bergen, Norway 1963/64 The objects of the

survey were several and included a study of the inter personal variation in the measurement of arterial B.P At the same time precautions were taken to reduce observer variation as much as possible

Outlay of the survey

Measurements of B.P weight and height were carried *out in connection with mass radiography of the chest at* several survey centres mostly schools in the city of Bergen The B P was measured last and the subjects were allocated at random among the observers Care was taken to rest the subject in a standardized position The initial pulse rate was noted In the subsequent auscultatory recording of the B P the following readings were taken

1 Systolic pressure at the first appearance of the Korotkow sound
2 Diastolic pressure 4th phase at the point of the muffling and
3 Diastolic pressure 5th phase at the point of disappearance of the sound

In a 10 per cent random sample of the entire population of Bergen 14 years and over chosen in advance of the study the special apparatus constructed by Rose Holland and Crowley model Mark 3 (7) was used in addition to the conventional sphygmomanometer for each subject The two instruments were used in a strictly alternating order from subject to subject and the same cuff was used for both instruments 14 by 40 cm

In all 70 445 subjects (42,550 females 27 895 males) 15–100 years of age had their B P measured The random sample consisted of 8?94 subjects examined (4658 females 3636 males)

Observers

The B P *was measured by registered nurses Among those* who volunteered for th project, 19 were accepted These 19 nurses had the following age grouping 20–29 years of age two 30–39 seven 40–49 five 50–59 four and 60–69 one

The nurses were thoroughly instructed during a training period of two weeks Basic information about the testing procedures was given and practical examinations were carried out under close supervision both on healthy subjects and on hospital patients In one nurse an audiogram was taken to test her hearing In addition, all the nurses were tested with a tape recording (7) of the sounds of 12 subjects with varying B P characteristics and with a pre fixed "true" reading of each compared *to a scale in seconds measured with a stop-watch Thus a* record of individual reading abilities was obtained which was later used for comparison both half way through the survey period and at the end of the survey

The study was planned so as to classify the results from each nurse according to the time of day stage of study period place of examination and age and sex of the subjects When both types of sphygmomanometer were used, these same details were noted and in addition the results from the two instruments were compared

Detailed printed instructions were given to each nurse In the first part of the study period the nurses were supervised to ensure that the instructions were adhered to and that no misunderstanding prevailed

Table I *The number of subjects examined by each observer*

Age groups 15–100 years

Observer	♂	♀	Total
A	2 207	3 258	5 465
B	647	1 261	1 908
C	750	1 347	2 097
D	1 941	2 972	4 913
E	2 190	2 880	5 070
F	1 359	3 187	4 546
G	2 083	3 162	5 245
H	1 103	1 644	2 747
I	2 050	3 264	5 314
J	839	1 284	2,123
K	1 316	1 918	3 234
L	2 066	2 936	5 002
M	668	892	1 560
N	1 269	1 586	2 855
O	2 376	3 486	5 862
P	753	1 333	2 086
R	973	1 385	2 358
S	1 131	1 567	2 698
T	2 174	3 188	5 362
Total	27 895	42 550	70 445

RESULTS

Blood pressure values among observers

The *total* results of the blood pressure readings for each observer were studied the systolic and the two diastolic pressures separately Considerable differences were found between the observers but much of this difference was due to the different age and sex composition of the groups examined by each nurse Consequently it was considered necessary to compare *sex and age specific values only*

Table I gives the number of subjects examined by each observer varying from 1560 to 5862

Here as well as in the figures and in the appendices the observers are identified by letters from A to T The position in the alphabet has been made on the basis of the total deviations by each observer from the group mean values of the systolic blood pressure of the different age groups

In appendices I–IV the mean values of the 3 blood pressures the standard deviations and the numbers examined are listed for each observer according to sex and age groups Only the ages 30–69 years are presented due to shortage of space (The results of the other age groups may be obtained from the authors) In these tables it may be seen that for *systolic* blood pressure some

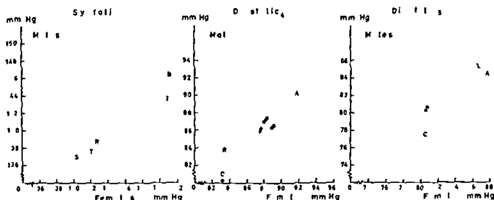


Fig 1 Correlation of mean blood pressure readings by each of 19 nurses in males and females age 50–59 years

of the observers have mean readings differing from the group mean value by as much as 5–11 mm Hg There is clearly a tendency for some observers to have readings higher than the group mean in all age and sex groups while others have low readings in all groups The difference between extreme observers is considerable Thus in the age group 60–69 years in females observer B has a mean reading of 171 5 mm Hg while observer S has 152 9 In the youngest group 20–29 years observer H has the highest reading in males of 132 5 mm Hg while observer S has 123 7

In the two *diastolic* pressures some of the observers deviate from the group mean by as much as 8–9 mm Hg but most of the deviations are between 0 and 4 mm Hg The greatest difference between extreme observers amounts to 95 2–81 7 mm Hg for observers P and C in diastolic 4th phase and to 88 7–74 8 mm Hg for observers A and C in diastolic 5th phase in the age group 70–79 years In the youngest age group in males the greatest difference in the 4th phase amounts to 81 0–67 5 mm Hg in observers H and O and in the 5th phase to 74 1–64 0 in observers L and J

It may be seen that in systolic readings the observers tend to keep the same *relative* order within the group of observers from age group to age group and between the sexes

In the diastolic readings the tendency to relative positioning within the group is different from the systolic and is not consistent to the same degree However as an example it may be mentioned that observer A read higher than the group mean

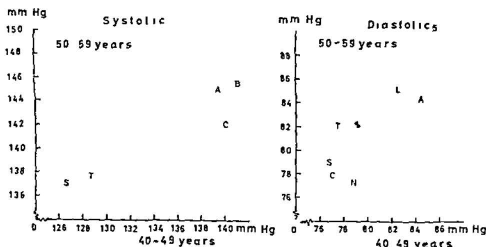


Fig 2 Correlation of mean blood pressure readings by each of 19 nurses in males age 40–49 and 50 59 years

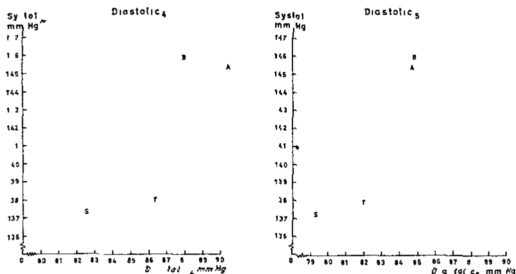


Fig 3 Correlation of mean systolic and diastolic readings by each of 19 nurses in males age 50–59 years

in all age groups for systolic as well as for the two diastolic pressures while observer S read lower in all three pressures Observer C read systolic pressure higher than the group mean while she read the two diastolic pressures lower than the group mean Observers F G I and M maintained an intermediate position in all readings

In Fig 1 the age group 50–59 years has been chosen to demonstrate the correlation between the readings in males and females for each observer The linear correlation coefficient between the mean values for each observer is 0 76 in systolic 0 89 in diastolic 4th phase and 0 85 in diastolic 5th phase pressure

Fig 2 shows that the reading of each observer in one age group correlates quite well with the readings in another age group of the same sex The linear correlation coefficient in males is 0 71 in systolic 0 70 in diastolic 5th phase pressure while in females the coefficient is higher 0 83 and 0 95

Fig 3 demonstrates that the readings of systolic and diastolic values are reasonably well correlated

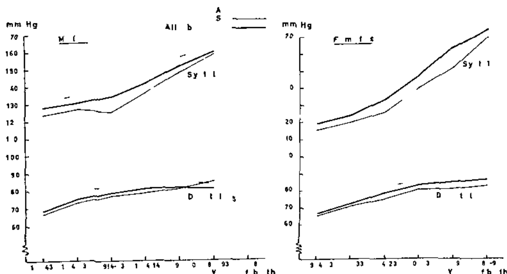


Fig 4 Systolic and diastolic blood pressure in age groups Mean values for observers A and S and for all observers

in each observer The correlation coefficient of systolic blood pressure to diastolic phase 4 is 0 68 and to diastolic phase 5 0 72 In females of the same age group the coefficients are 0 65 and 0 72 respectively

The difference between extreme observers A and S is illustrated in Fig 4 where the mean values of systolic and diastolic 5th phase pressure are compared with those of the total of the 19 observers

The variation of blood pressure readings in each observer

The variation of the readings in each observer is indicated by the size of the standard deviation in each age and sex group for the 3 blood pressures which may be seen in appendices I–IV No definite trend surges to indicate a real reading differences between the observers

Even if differences between the observers regarding variation of the readings *may* be partly caused by actual *reading* differences they may also be influenced by the variations in the actual pressures between the subjects examined Consequently in this material a closer study of the standard deviation does not form sufficient basis for differentiation between observers as regards reading stability

The influence of the sex of the subject examined

In appendices I–IV it may be seen that in each observer the magnitude of the difference from the group mean value is about the same for males as for females Fig 1 also indicates no great difference between the readings in the two sexes There is therefore no tendency to obtain different readings in males from those in females and there is no indication of influence of age charm or personality of the female observers This point will be studied below using a different approach

Terminal digit preference

Preference for certain terminal digits in the readings by the different observers has been investigated In *the total group* of observers a small preference is indicated for zero values in the use of the *conventional* apparatus to measure *systolic* blood pressure

Digits	0	2	4	6	8
Percentage	25	18	20	17	20

When the special apparatus of Rose Holland and Crowley is used the digit preference is much less

Digits	0	1	2	3	4	5	6	7	8	9
Percentage	14	8	11	8	10	9	11	8	12	8

In this apparatus however the preference for the zero value is also indicated and the uneven digits are slightly underrepresented

In the individual observers appendices V and VI demonstrate a much greater variation in digit preference Observers L and S have a definite preference for zero in the use of the conventional apparatus Observers B H J and O show the same preference to a lesser extent In the others no definite digit preference is found Oddly enough some of the observers also show some digit preference in using the special apparatus This is particularly so for observers D and S and to a lesser degree for observers K M and R

The tendency demonstrated here for the systolic blood pressure is also present for the diastolic 4th phase In appendix VI it may be seen that observers D and S have preferred the even digits in the use of the special apparatus

Differences between results according to type of apparatus used

In the 10 per cent random sample of the adult Bergen population the two types of blood pressure apparatus used for each subject allow for several comparisons of the reading characteristics of each observer The special apparatus is supposed to give readings with less reader bias such as terminal digit preference and overestimation of certain values Thus the results of the special apparatus may be used as an indication of the "true" blood pressure at the particular moment and under the particular circumstances Consequently the difference between the results obtained with the two apparatuses may give an indication of the accuracy of the reading when the conventional apparatus has been used

However the chosen procedure of alternatively using the special (S) or the conventional, ordinary (O) apparatus first influences the results The time necessary for the first reading represents a period of rest, which in turn may give a lower reading with the second apparatus This period of rest may be longer when the more com

Fig 5 Comparison of differences between systolic blood pressure measured in the first and the second half of the work period each day with the two apparatuses in the same individuals—observers A and G

plicated S apparatus is used first In addition to such a rest factor an apparatus factor may be present because the S apparatus tends to give lower systolic readings than the O apparatus

The difference between the results when either the S or the O apparatus is used first may be listed

O_1-S apparatus factor plus rest factor (which is small short time)

O_2-S apparatus factor minus rest factor (which may be longer than the above due to longer rest period)

Consequently the differences between the results of the two apparatuses will be greatest when the ordinary conventional apparatus is used first and the special apparatus second As the rest factor is supposedly of greater importance in systolic than in diastolic blood pressure the difference between the two differences listed above would be expected to be greatest in systolic values

Several of the possible reader characteristics have been studied by using the differences between the results of the two apparatuses just mentioned

Influence of sex

In the majority of observers the influence of the sex of the individual examined is found to be insignificant using this method of investigation In observer N, the males have greater apparatus differences than the females—approximately 5 mm Hg for the systolic 3 mm Hg for the diastolic values In observer F the females have greater differences than the males but only approximately 2 mm Hg

Thus also using this method of study the majority of observers do not differ much from each other whether they measure males or females The sex component seems altogether to be a very small part of the experimental error

Influence of age

The importance of the age of the subject examined has been analysed in the same way among the observers There is no difference in this respect between the results of the two apparatuses used Consequently it may be assumed that no systematic error depending upon the age of the subjects examined exists among the observers

Influence of fatigue

The same method has been used to study the effect of fatigue on the reading of blood pressure by individual observers during their working period which lasted 3–6 hours A rest of 15 minutes was taken whenever this was felt necessary by the observer and when the conditions permitted The difference between the mean results of the two apparatuses in the first and second half of the daily working period has been used for this investigation Practically no difference is found between the results from the two periods Fig 5 gives an illustration of this comparing the frequency distributions of the differences in two particular observers A and G

Thus the reading characteristics do not seem to have been influenced much by fatigue in the observers (Each observer examined between 1560

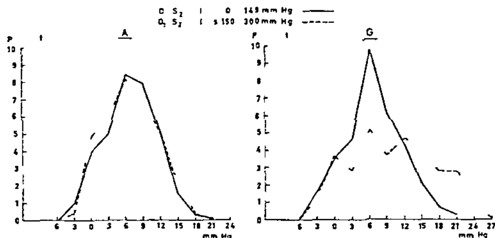


Fig 6 Comparison of differences between systolic blood pressure measured with the two apparatuses in the same individuals grouped in values above and below 150 mm Hg—observers A and G

and 5862 subjects during the study period of approximately 4 1/2 months giving a number of 20-50 examinations per day In one hour the number of examinations per observer was 1 to 15 (with the greatest load occurring during particular "rush" hours)

Influence of the height of the blood pressure

The differences between observers in reading characteristics may depend upon the height of the blood pressure measured Differences between the results of the two apparatuses were therefore studied with a systolic blood pressure grouping of less than 150 mm Hg compared to 150 mm Hg and above In this analysis no great influence of the height of the blood pressure in this respect was demonstrated either in the individual observer or between observers As an illustration of the comparisons carried out, Fig 6 gives the frequency distributions of the differences between the values obtained with the two apparatuses in the same subjects comparing observers A and G It may be seen that observer A demonstrates almost identical curves for the two blood pressure levels The same was found for the majority of the other observers as well. Observer G clearly shows different distributions in the two pressure levels This was also found when she used the special apparatus first.

The reason why observer G had relatively large differences between the results of the two apparatuses in her high pressure group may be that she used a long time for her examination giving some of the "artificially" high pressures time to decrease in the second reading Chance factors may also have been involved and she may have had particularly large numbers of subjects with high but labile systolic blood pressure

Altogether the differences and similarities between the 19 observers illustrated above may partly depend upon individual reading characteristics but may also of course be a result of true differences between the subgroups examined by each observer The latter factor may be reduced by the large number of subjects examined and the use of the mean values specific for sex and an age grouping of ten years But this factor of "true" differences between the subjects measured cannot be disregarded

The ideal method for comparing the observers would be to let them examine the same subjects under standardized conditions This ideal was aimed at in the application of the tape recording test the results of which are reported in (4)

DISCUSSION

The Bergen Blood Pressure Survey of 1963/64 has covered a group of nearly 70 000 subjects in 4 1/2 months Such a number of blood pressure readings in so short a period requires a large team of observers Variations in reading tech

nique and in individual factors may produce differences between the observers It may be of interest to study these differences and their influences upon the total results

First of all the selection of observers for the blood pressure examinations is important. In the Bergen survey qualified nurses were chosen The reason for this was partly the success of using nurses in the survey of 1950/51 (1) and partly the impossibility of getting sufficient numbers of physicians to carry out the work Several surveys have utilized nurses or technicians for the measurement of blood pressure (1 2) and their performance has been considered as good as if not better than that of physicians (3 7)

In the present study two of the nurses who volunteered for the examinations were more than 55 years of age In one of these it was found necessary to have an audiogram taken to ensure that her acoustic sense was adequate Otherwise the two older nurses were efficient enough during the testing and training period to warrant their inclusion in the team. During the survey the older nurses had somewhat greater difficulties than the younger ones during rush hours with many examinations That they showed no influence of fatigue however may be explained by the routine rest period and perhaps particular care shown by their colleagues

The analysis of the results of these two nurses observers L and N showed that observer L tended to have very high readings of diastolic 5th phase pressure while she had intermediate systolic and diastolic 4th phase readings This may be associated with her slightly deficient hearing Observer N tended to have very low diastolic values which may be explained by good hearing or long reaction time The latter explanation is supported by her results in the tape recording test

However the two older nurses are not significantly different from the others in their total accomplishment. Among the other nurses too facility for blood pressure reading could not be graded on the basis of age in the findings of the present survey Otherwise none of the nurses had had any great experience in blood pressure measurement before the survey Consequently their abilities had to be judged during their period of training the training had to be very thorough and detailed as were the written instructions

Even when sex and age specific values were used the total results demonstrated some differences between the observers In systolic pressure the greatest difference was to be found between observers B and S in females aged 60–69 years This difference amounted to 18 6 mm Hg In diastolic$_4$ pressure the greatest difference was found between observers P and C in females aged 70–79 years where it was 13 5 mm Hg In diastolic pressure the greatest difference was between observers A and C in females aged 70–79 years and was 13 9 mm Hg These differences between these particular observers were 5–6 times the standard error of the difference between means Apart from the extreme observers the differences between observers and between individual observers and the group mean were less than twice the standard error

Of course the composition of each sex and age specific group examined by each observer may influence the mean values and explain the inter observer differences But when these differences are of the same size and direction from age group to age group in both sexes one may assume that there is an actual reading variation between observers Consequently there is justification in studying the individual observer characteristics concerning blood pressure reading by comparing sex and age specific values Thus a ranking list may be made out in which the position of any particular observer may be studied for the 3 blood pressures

Observer A is top of this list reading all 3 pressures higher than her colleagues Observer S is at the bottom when all 3 pressures are taken as a whole—even if the positions at the bottom change somewhat from one pressure to the other The rest of the observers occupy partly the same intermediate ranking positions but some have also been found to vary considerably between the 3 pressures This reading variation between the observers influences the mean values of the total results but with the great numbers examined in the present study there is no distortion of the distribution curves

Other reading characteristics are demonstrated in this survey The terminal digit preference is small when the total results for all observers are investigated But when the totals are split according to each observer definite differences appear The preference of the zero value is clearly present in this study as in so many others but

the zero does not dominate to the same extent as in other reports (1 2 5 7)

In those observers where terminal digit preference is pronounced it is consistent in all 3 blood pressures It might be expected that the systolic reading with its quite definite hearing point would be less liable to this bias than the 2 diastolic readings but this is not so in the present study

The special sphygmomanometer of Rose Holland and Crowley clearly reduces the reading errors represented by the terminal digit preference but in three observers in the present study D P and S preference for even digits is obvious Observer S also has a pronounced zero-preference in the use of the conventional apparatus while the preference of observer D is mainly present in the use of the special apparatus. But here it is consistent in all 3 pressures

Owing to the construction of the special apparatus it is very surprising that these results of observers D P and S should occur In observer D only 16% of the readings have an uneven terminal digit in observer S 9%. The number of readings should be large enough to reduce the risk of pure chance results of that kind The explanation may be that a "writing bias has been introduced. But a closer study shows that this bias must have been present when the special apparatus was used first as well as second and at all times of the day and at all blood pressure levels It *may* be that the bias arises because of the combination with the measurements of the conventional apparatus where only even digits are present. In any case it must be assumed that the observers D P and S have not followed the detailed printed instructions for the reading and subsequent writing of the blood pressure measured with the special apparatus

The part played by fatigue in the experimental error in blood pressure measurement under population survey conditions does not seem to have been great in the present material Some explanation of this point may be found in the design of the survey The number of observers in relation to the number of subjects to be examined the routine of alternating work and rest the number of hours at work per day and all the other details of the work were planned on the basis of careful calculations and considerations—in order to avoid too much stress upon the observer nurses (and simultaneously upon the subjects to be examined) That no significant differences may be found between the reading results from the two halves of the daily working period may be taken as an indication that these efforts at fatigue prevention were successful

The influence of the age and sex of the subjects examined and the level of the blood pressure measured have not had much effect on the reading error These factors have partly been studied with the aid of the differences between the readings with the two sphygmomanometers used but this study basis may be ill suited for the purpose However the care taken in the blood pressure readings the relatively great number of readings and the consistent findings in this respect for all 3 blood pressures give support to the conclusions

The use of the two sphygmomanometers in the 10 per cent random sample of the adult Bergen population as a part of the present study has been of importance in several ways The apparatus factor thus introduced has made possible a correction of the readings with the conventional apparatus At the same time the observer nurses became interested in the readings and were anxious to avoid faults These conditions may however have introduced a factor of bias in the reading

The terminal digit preference has been definitely reduced in the use of the special apparatus as previously mentioned Pressing of the three buttons has not presented any problems and the inspection of the mercury columns and levelling of the cursors to the meniscus have had the advantage of giving sufficient time for accurate readings The constant rate of deflation of the cuff pressure was an important part of the standardized procedure (7)

The use of an additional apparatus has required additional time and usually rest for the subject to be examined Consequently this procedure itself may give lower blood pressure values especially for the systolic pressure Thus the blood pressure findings will be somewhat lowered in the group in which both instruments were used This will affect the mean values and will place the frequency distribution slightly more on the side of the lower values than if only one apparatus had been used This point however has no influence upon the problem of the particular study here presented

The results of the differences between the mean

values of systolic and diastolic blood pressures of the individual observers in this series agree quite well with the findings of Lowe and McKeown (5) These authors found consistent and substantial differences between readings by 12 physicians of approximately 12 mm Hg between the highest and the lowest readings

When comparing the mean deviations of each observer in this series with those found by Comstock (2) the mean difference in systolic pressure is found to be of almost the same magnitude except for observers R and T and partly A and B Further comparisons are not being made because most other studies do not give a relevant basis for such comparison

REFERENCES

1 Bøe J Humerfelt S & Wedervang, F The blood pressure in a population Acta med scand Suppl 321 1957
2 Comstock, G W An epidemiologic study of blood pressure levels in a biracial community in the southern United States Amer J Hyg 65 271 1957
3 Doyle J T Criteria for diagnosis of disease and clinical evaluation methodology epidemiology of cardiovascular diseases Amer J publ Hlth Suppl 20 1960
4 Eilertsen E & Humerfelt S Objective method for evaluation of observer variation in blood pressure reading Acta med scand In press
5 Lowe C R & McKeown T Some sources of irregularity in the distribution of arterial pressure In Epidemiology p 131 J Pemberton London 1963
6 Pickering, G The nature of essential hypertension, p 61 Churchill London 1961
7 Rose G A., Holland W W & Crowley E A A sphygmomanometer for epidemiologists Lancet 1 296 1964
8 Smirk F H High arterial pressure p 32 Blackwell Oxford 1957
9 WHO Hypertension and coronary heart disease Classification and criteria for epidemiological studies Wld Hlth Org techn Rep Ser 168 1959

Appendix I *Numbers examined (n) mean blood pressure (x) and standard deviation (s) in males and females born 1894–1903 per observer*

Males

Observer	n	Systolic x	Systolic s	Diastolic$_4$ x	Diastolic$_4$ s	Diastolic$_5$ x	Diastolic$_5$ s
A	297	158.5	28.3	90.1	15.0	84.2	15.0
B	81	156.8	28.5	87.8	13.5	84.5	13.6
C	9[illegible]	162.2	27.3	84.7	14.2	80.7	15.6
D	[illegible]57	153.4	27.0	86.5	13.6	81.9	14.2
E	301	154.0	25.8	86.1	12.4	80.3	12.5
F	291	154.1	27.6	86.7	13.5	82.4	13.8
G	30[illegible]	154.5	26.3	87.2	14.0	81.3	14.6
H	218	156.9	27.6	89.9	12.9	84.8	13.2
I	291	153.6	27.5	86.8	14.5	81.8	14.7
J	81	153.0	27.0	87.4	12.7	81.1	12.4
K	260	154.3	27.7	84.8	12.0	80.4	12.7
L	313	152.4	24.0	87.3	13.2	85.4	13.6
M	97	154.9	31.1	86.5	14.4	83.7	13.9
N	272	154.0	27.5	84.1	13.5	77.5	14.7
O	338	155.7	27.5	86.0	13.2	82.1	13.6
P	117	146.8	[illegible]2.1	87.5	12.3	83.0	12.5
R	119	146.3	22.5	83.2	10.6	80.5	11.0
S	116	148.7	25.9	85.8	13.0	82.5	13.5
T	303	144.0	23.1	86.4	12.8	82.3	13.4
Total	4146	153.5		86.7		8[illegible].2	

Females

Observer	n	Systolic x	Systolic s	Diastolic$_4$ x	Diastolic$_4$ s	Diastolic$_5$ x	Diastolic$_5$ s
A	50[illegible]	169.9	30.9	93.4	13.7	88.8	14.2
B	155	171.5	29.0	91.2	14.1	86.8	15.7
C	170	16[illegible].4	27.4	82.7	14.5	79.6	14.6
D	409	167.3	31.0	9[illegible].6	14.3	86.[illegible]	14.5
E	397	164.5	[illegible]7.9	89.6	13.7	83.1	13.4
F	509	169.5	29.9	9[illegible].9	14.2	88.3	14.5
G	438	167.2	29.8	90.5	14.5	83.4	14.7
H	380	164.1	28.5	91.3	11.6	85.8	12.4
I	501	165.1	[illegible]9.8	90.0	13.2	84.6	13.4
J	159	165.4	30.7	90.7	14.4	83.7	15.5
K	497	166.8	30.7	88.3	13.0	83.3	13.5
L	478	161.9	26.7	90.4	13.2	88.4	13.7
M	105	158.3	26.8	86.1	13.1	81.7	15.7
N	359	163.0	31.4	86.4	13.3	79.5	13.3
O	517	165.3	31.8	89.1	13.8	85.1	13.8
P	210	164.6	26.4	93.7	13.9	87.6	15.0
R	144	165.1	31.3	89.3	14.9	85.0	14.7
S	188	152.9	27.9	84.7	13.4	81.2	13.4
T	465	156.9	29.2	91.5	14.0	86.3	15.6
Total	6583	164.8		90.2		85.2	

Appendix II *Numbers examined (n) mean blood pressure (x) and standard deviation (s) in males and females born 1904–1913 per observer*

Males

Observer	n	Systolic x	Systolic s	Diastolic$_4$ x	Diastolic$_4$ s	Diastolic x	Diastolic s
A	381	145.5	22.9	90.1	15.0	84.4	12.7
B	1[illegible]8	146.0	[illegible]8.5	87.6	10.8	84.4	11.4
C	138	14[illegible].3	21.8	80.5	11.4	78.0	11.3
D	336	145.2	24.4	86.5	13.6	83.4	13.0
E	376	143.8	[illegible]1.8	86.1	12.4	80.4	11.8
F	246	145.4	22.4	86.4	11.7	82.0	12.6
G	382	145.6	24.0	87.2	13.5	8[illegible].1	13.5
H	190	143.8	[illegible]3.3	87.3	11.6	82.9	11.4
I	415	143.8	23.3	85.6	10.8	81.1	11.1
J	135	145.2	22.8	85.9	10.8	79.3	12.1
K	[illegible]29	143.1	22.6	84.8	11.0	80.6	11.8
L	356	144.6	21.2	87.0	11.9	85.1	13.6
M	118	145.0	22.3	85.7	11.3	84.1	11.3
N	235	141.0	2[illegible].6	82.8	11.7	77.6	11.6
O	421	139.0	[illegible]1.5	8[illegible].6	11.2	79.7	11.9
P	143	143.0	24.9	87.6	13.3	82.2	13.7
R	173	138.6	[illegible]3.8	83.5	11.9	81.[illegible]	11.6
S	189	137.5	[illegible]0.2	8[illegible]	10.1	79.0	10.4
T	364	138[illegible]	[illegible]2.6	86.0	12.6	81.7	12.5
Total	4955	14[illegible].9		85.7		81.7	

Females

Observer	n	Systolic x	Systolic s	Diastolic x	Diastolic s	Diastolic x	Diastolic s
A	566	15[illegible].0	[illegible]6.3	91.3	13.0	87.2	12.9
B	211	151.8	24.8	87.9	11.7	84.0	12.0
C	2[illegible]3	149.3	21.3	83.4	11.1	80.3	11.8
D	492	149.2	25.5	89.1	12.2	83.8	1[illegible].3
E	495	149.4	25.7	87.7	12.9	81.8	13.1
F	434	148.7	[illegible]7.2	89.0	12.0	85.0	12.1
G	581	148.8	[illegible]5.5	88.2	12.7	83.0	13.2
H	308	148.9	26.4	88.3	10.8	84.4	11.3
I	539	150.5	[illegible]6.6	88.0	12.4	83.4	12.7
J	219	145.9	25.2	87.1	12.7	81.8	13.1
K	35[illegible]	146.8	[illegible]5.8	84.8	11.3	80.9	11.7
L	503	146.4	[illegible]3.7	88.1	13.0	86.2	13.3
M	151	145.1	22.0	84.9	11.5	83.1	12.1
N	349	147.2	[illegible]5.5	84.7	12.[illegible]	79.1	11.9
O	685	146.6	25.1	86.0	1[illegible].0	8[illegible].8	12.2
P	233	145.2	[illegible]4.6	89.7	13.1	84.9	1[illegible].3
R	[illegible]3	14[illegible].1	23.7	84.1	11.1	81.1	11.4
S	277	140.4	23.4	84.1	12.6	80.7	1[illegible].5
T	541	141.9	[illegible]6.3	87.6	1[illegible].7	83.4	12.9
Total	7382	147.5		87.4		83.3	

Appendix III *Numbers examined (n) mean blood pressure (x) and standard deviation (s) in males and females born 1914–1923 per observer*

	Males							Females						
		Systolic		Diastolic$_4$		Diastolic			Systolic		Diastolic$_4$		Diastolic$_5$	
Observer	*n*	*x*	*s*	*x*	*s*	*x*	*s*	*n*	*x*	*s*	*x*	*s*	*x*	*s*
A	449	139 3	19 0	88 3	11 7	83 8	12 2	571	137 3	21 2	85 5	11 6	81 7	12.2
B	113	140 7	18 2	85 6	10 9	81 3	11 5	190	138 3	21 0	82 9	11 4	79 4	11 8
C	*130*	*139 6*	*17 3*	*80 5*	*11 1*	77 6	*11 6*	*195*	*134 4*	*18 6*	77 5	10 4	75 2	10 9
D	393	134 9	17 3	85 4	10 3	80 8	10 8	565	134 8	20 6	84 0	11 2	79 0	11 6
E	476	135 7	17 7	83 3	10 5	77 6	11 0	554	136 3	21 3	82 9	11 4	77 5	11 9
F	191	135 7	20 0	85 6	12 1	81 1	12 4	323	133 7	17 8	84 5	10 5	80 4	10 9
G	380	136 6	18 5	84 6	11 3	79 2	11 8	570	135 9	20 6	82 9	11 0	78 2	12 0
H	*151*	*137 2*	*20 9*	*86 8*	*10 8*	*81 7*	*11 3*	*189*	*133 1*	*18 2*	83 7	10 1	79 4	10 5
I	420	136 7	18 6	84 2	10 1	79 5	10 4	579	133 8	18 7	82 7	10 1	78 4	10 3
J	145	134 9	17 1	85 1	10 5	78 5	12 3	186	134 8	19 [illegible]	83 7	9 9	77 1	11 5
K	206	131 8	16 0	80 4	9 1	76 2	9 6	277	135 8	21 0	81 5	11 1	77 2	11 2
L	381	136 4	16 4	84 0	10 4	82 0	10 6	528	133 4	18 3	83 0	11 2	81 0	11 4
M	153	135 1	18 4	81 7	11 1	77 8	13 8	160	135 3	21 7	81 2	12 3	78 3	13 1
N	163	136 9	19 6	82 9	11 2	78 1	11 2	207	132 7	21 0	80 2	11 3	75 6	11 3
O	430	131 9	19 2	80 8	10 7	77 3	11 4	602	132 1	20 6	80 2	10 9	76 9	11 1
P	140	131 6	15 6	83 9	9 1	79 1	10 8	242	13[illegible] 1	19 1	84 7	11 4	80 3	12 4
R	205	132 5	17 5	80 7	10 3	77 2	10 5	279	131 5	18 0	80 2	10 2	77 7	10 4
S	169	129 6	16 4	80 3	10 1	77 4	10 4	221	126 4	18 2	78 6	11 3	75 0	11 6
T	411	1[illegible]8 6	19 0	82 7	10 6	77 8	10 8	567	128 7	20 5	82 6	11 5	78 1	12 3
Total	5056	134 9		83 7		79 3		7005	133 8		82 6		78 5	

Appendix IV *Numbers examined (n) mean blood pressure (x) and standard deviation (s) in males and females born 1924–1933 per observer*

	Males							Females						
		Systolic		Diastolic$_4$		Diastolic$_5$			Systolic		Diastolic$_4$		Diastolic$_5$	
Observer	*n*	*x*	*s*	*x*	*s*	*x*	*s*	*n*	*x*	*s*	*x*	*s*	*x*	*s*
A	336	135 6	15 8	84 7	10 6	79 3	11 1	452	129 3	15 4	81 [illegible]	10 3	76 9	11 1
B	92	134 0	13 3	82 3	10 1	77 4	11 9	140	126 1	16 4	76 9	9 4	72 9	10 0
C	116	132 8	13 7	73 3	11 5	69 8	12 0	156	125 2	14 3	71 8	9 8	69 2	10 3
D	311	132 4	16 7	82 5	11 2	77 2	1[illegible] 4	496	125 3	16 6	78 4	10 2	7[illegible] 9	10 9
E	36[illegible]	131 5	15	79 9	9 9	73 1	10 6	437	125 2	14 2	76 0	9 3	70 4	10 0
F	133	129 0	15 9	79 7	10 0	75 2	10 4	197	126 0	15 8	79 3	10 1	74 9	10 1
G	320	131 0	14 3	80 0	9 6	73 4	11 1	489	125 1	15 1	76 7	9 8	71 3	10 9
H	127	130 6	14 3	83 2	8 4	77 2	9 5	180	125 8	14 3	79 8	8 7	74 1	9 4
I	29[illegible]	132 3	14 2	80 0	8 4	74 9	10 4	464	126 0	14 6	77 9	10 1	73 2	10 6
J	147	129 9	14 8	79 8	8 7	71 6	10 7	144	124 9	15 7	77 4	9 6	70 9	11 1
K	150	129 6	15 4	77 3	9 5	72 5	10 2	158	126 3	14 1	75 3	8 8	71 0	10 7
L	292	134 1	14 7	80 9	11 3	78 8	11 6	431	126 0	12 7	77 6	9 3	75 4	9 4
M	95	1[illegible]9 8	13 6	78 1	9 8	75 1	10 8	133	123 9	14 2	73 5	9 3	70 5	10 5
N	*136*	*130 5*	*13 7*	78 6	9 6	72 6	10 6	138	121 7	13 0	74 8	8 7	69 6	10 [illegible]
O	360	1[illegible]8 1	14 8	78 0	9 2	74 2	9 8	522	122 3	14 4	75 1	9 2	71 4	10 1
P	121	1[illegible]0 7	14 1	82 0	9 4	76 4	10 6	215	124 8	14 6	79 3	10 5	75 1	11 0
R	168	128 8	13 2	77 8	10 0	74 8	10 1	238	12[illegible] 4	13 7	74 7	9 4	71 1	9 6
S	183	127 5	15 0	76 6	11 9	73 5	11 9	191	120 5	13 7	74 5	10 5	71 5	10 6
T	[illegible]54	127 3	16 6	80 7	10 1	75 9	11 5	469	118 2	15 1	76 3	10 1	71 2	12 0
Total	4095	130 9		80 1		75 2		5650	124 5		77 0		7[illegible] 6	

Appendix V *Distribution of terminal digits in the readings of each of 19 observers with the conventional and the special apparatus*

Systolic blood pressure

Ob server	Conventional apparatus ( )					Special apparatus ( )									
	0	2	4	6	8	0	1	2	3	4	5	6	7	8	9
A	25	13	20	23	19	14	9	11	11	10	8	8	7	12	10
B	28	16	19	21	16	14	6	6	5	9	10	15	14	14	7
C	17	26	19	17	21	9	6	12	8	13	10	9	9	14	10
D	15	18	27	16	24	15	2	18	3	17	2	17	2	2[illegible]	2
E	26	24	22	13	15	9	9	8	8	10	12	11	11	12	10
F	15	25	18	22	20	13	9	11	10	9	9	10	8	11	10
G	15	20	21	24	0	11	12	9	8	12	8	11	10	10	9
H	31	15	20	14	20	21	7	14	5	10	10	10	7	10	6
I	19	20	20	19	22	12	11	9	9	6	12	10	10	10	11
J	31	15	17	15	22	10	7	12	8	12	12	11	8	11	9
K	13	24	20	0	23	3	20	8	14	4	13	4	14	8	12
L	42	16	17	1[illegible]	13	18	7	8	8	9	9	9	10	11	11
M	24	14	22	17	23	23	6	10	9	8	3	13	8	15	5
N	28	17	23	18	14	27	5	12	6	11	10	10	4	11	4
O	29	19	21	12	19	11	7	12	9	12	10	10	10	11	8
P	23	19	21	19	18	15	6	17	7	12	8	13	9	16	7
S	56	13	6	4	21	27	2	11	2	10	3	17	5	18	5
T	18	18	17	22	25	16	6	13	7	12	9	10	8	12	5
Total	25	18	20	17	20	14	8	11	8	10	9	11	8	12	8

Appendix VI *Distribution of terminal digits in the readings of each of 19 observers with the conventional and the special apparatus*

Diastolic$_4$ blood pressure

Ob server	Conventional apparatus ( )					Special apparatus ( )									
	0	[illegible]	4	6	8	0	1	2	3	4	5	6	7	8	9
A	28	16	20	23	13	10	9	10	10	9	10	12	9	12	11
B	33	16	20	15	16	9	13	11	8	12	9	9	11	9	9
C	30	16	20	20	14	11	8	9	8	14	10	9	10	14	7
D	21	16	1	14	25	16	2	14	2	17	3	17	4	20	5
E	38	17	17	13	15	9	6	10	9	10	9	12	11	10	14
F	28	17	17	15	23	7	8	9	11	10	11	10	9	11	14
G	20	16	23	23	18	12	9	9	11	10	9	8	12	10	10
H	46	8	18	14	14	13	6	11	10	10	9	10	1[illegible]	12	7
I	22	14	22	20	22	10	12	9	8	7	9	10	11	13	11
J	37	16	24	12	11	10	11	10	10	11	12	8	9	10	9
K	30	10	20	25	15	10	12	3	13	13	8	11	10	8	12
L	29	16	20	14	11	14	6	13	10	10	1[illegible]	7	10	8	10
M	22	21	27	18	12	16	6	14	7	9	7	14	6	11	10
N	48	16	2[illegible]	7	7	18	7	9	10	12	9	9	7	13	6
O	33	16	20	9	22	12	9	14	6	9	9	10	11	9	11
P	39	13	2[illegible]	16	10	18	2	15	9	16	7	12	3	15	3
S	50	12	19	7	12	23	1	12	1	15	2	21	2	0	3
T	15	26	19	14	26	13	7	10	8	12	13	10	9	10	8
Total	30	17	[illegible]0	16	17	12	8	11	8	11	9	11	9	12	9

Table I *Incorporation of* DL *mevalonate 2* ¹⁴C *and* DL *leucine 4 5* ³*H into serum free cholesterol in normo and hypercholesterolemic human subjects*

Case no	Age (y)	Body weight (kg)	Serum tri glycer ides (mg/100 ml)	Serum cholesterol: Total (mg/100 ml)	Serum cholesterol: Free (mg/100 ml)	Serum cholesterol: Pool size (g[a])	Free ¹⁴C-cholesterol: Injected dose at peak SA[b] ( )	Free ¹⁴C-cholesterol: SA ratios (½ h/peak)	Free ¹⁴C-cholesterol: SA ratios (48 h/peak)	Free ³H cholesterol: Injected dose at peak SA[b] ( )	Free ³H cholesterol: SA ratios (½ h/peak)	Free ³H cholesterol: SA ratios (48 h/peak)
1	61	58 0	226	640	201	5 25	7 28	0 064	0 406	0 1350	0 039	0 338
2	35	64 0	244	539	130	3 75	2 61	0 109	0 343	0 0 03	0 448	0 375
3	31	72 0	100	310	104	3 37	3 41	—	0 324	0 0167	—	0 443
4	28	68 0	100	500	167	5 11	7 57	0 154	0 404	0 1170	0 175	0 251
5	51	80 5	500	504	141	5 11	6 10	0 049	0 407	0 0291	0 172	0 446
6	41	76 5	152	261	72	2 48	3 91	0 063	0 238	0 0332	0 496	0 714
7	43	66 0	75	234	57	1 69	2 55	0 137	0 288	0 0274	0 241	0 347
Mean			200	427	125	3 82	4 78	0 096	0 344	0 0541	0 262	0 416
±S E							±0 82	±0 01	±0 025	±0 0188	±0 072	±0 056

[a] Plasma volume was assumed to be 4 5 of body weight
[b] SA = specific activity (dpm/mg)

C (both used as produced by The Radiochemical Centre Amersham England) was prepared in dilute ethanol and a known amount (80 μC of H and 10 μC of C) was administered intravenously Blood samples were then obtained / 1 2 4 8 24 and 48 h later Serum was separated and kept frozen before analysis Extraction of lipids was carried out by 10 volumes of ethanol ether (1 ?) Esterified cholesterol was separated from free cholesterol and triglycerides by thin layer chromatography on silica gel G by developing the chromatoplates with heptane ethyl ether acetic acid (80 20 1) After extraction of lipid classes fatty acids of the cholesterol ester fraction and of the free cholesterol fraction (containing diglycerides) were removed by saponification for 1 h at 45 C in ethanolic (90 %) NaOH (1 *M*) solution Water was added to bring ethanol concentration to 60 and cholesterol was extracted with petroleum ether Cholesterol was measured according to Hansen and Dam (9) and triglycerides by the method of Carlson (?) Radioactivity was counted in a Packard Tri-Carb Liquid Scintillation Spectrometer using external standard for the *determination of the absolute activity levels (dpm)* Serum α ketoisocaproate level was measured according to DeSchepper et al (5)

The fractional turnover rate of esterified cholesterol was calculated both for ³H and C cholesterol by plotting the specific activities of the free and esterified cholesterol on cartesian graphpaper The calculations were carried out as presented by Zilversmit (?4) assuming that serum esterified cholesterol is derived from free cholesterol in the plasma or liver The validity of the method for the turnover rate of serum esterified cholesterol is discussed in detail by Nestel and Monger (19) They assumed that the small amount of esterified cholesterol present in the liver (less than 10 of the plasma pool) causes an insignificant error in the results

RESULTS

Incorporation of ¹⁴C mevalonate and ³H leucine into serum free cholesterol

Table I shows that up to 8 % of injected DL mevalonate 2 ¹⁴C and only from 0 017 to 0 135 % of DL leucine-4 5 ³H were converted to serum free cholesterol at its peak specific activity The plotting of these values against the pool size of free cholesterol according to Fig 1 demonstrates particularly for ¹⁴C cholesterol that the larger the serum pool the more of the label is found in serum free cholesterol

That the rate of the appearance of the two labels in serum free cholesterol is not the same is illustrated by the ratio ¹⁴C/³H in Fig 2 The ratio increases rapidly reaching its maximum at 4 h and falls thereafter slowly This suggests that shortly after administration of the labels

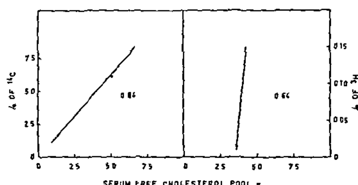


Fig 1 Correlation of the serum free cholesterol pool to the radioactivity (of dose) found in serum free cholesterol at the peak specific activity after simultaneous administration of DL mevalonate ^{14}C (left side) or DL leucine-4,5 H (right side) intravenously to human subjects

cholesterol synthetized from leucine entered into the circulation faster than that formed from mevalonate A slow decrease of the ratio after 4 h (at 48 h all values were lower than at 4 h) indicates on the other hand that the release of ^{3}H-cholesterol into the circulation is more prolonged than that of ^{14}C-cholesterol This may be due to recirculation of ^{3}H-compounds derived from ^{3}H leucine or release of ^{3}H-cholesterol from tissues which synthetized less ^{14}C-cholesterol The faster rate of increase and the slower rate of fall of the specific activity of H cholesterol as compared to ^{14}C cholesterol is seen also in the ratios $^{1}/$ h and 48 h to the peak specific activity (Table I) These ratios for ^{3}H-cholesterol were in general higher than those for ^{14}C-cholesterol Furthermore the rate of fall of the specific activity as indicated by the ratio of the 48 h to the peak specific activity showed an inverse correlation to the pool size of serum free cholesterol in the case of ^{14}C ($r = 0\,90$) but not in the case of ^{3}H ($r = -0\,44$)

Role of serum α ketoisocaproate in synthesis of ^{3}H-cholesterol from ^{3}H leucine

The relatively low conversion of ^{3}H leucine to cholesterol as compared to that of ^{14}C mevalonate and almost tenfold variation from case to case could have been due to different dilution of the precursor into a large serum or tissue pool of leucine or its metabolites Therefore α ketoisocaproate the first metabolite during the conversion of leucine to HMG was quantitated in serum Table II shows that the values vary between 0 30 and 0 91 mg/100 ml However there was no correlation to the respective peak specific activities of serum free ^{3}H-cholesterol suggesting that ^{3}H-cholesterol synthesis was not dependent on the α ketoisocaproate concentration Further

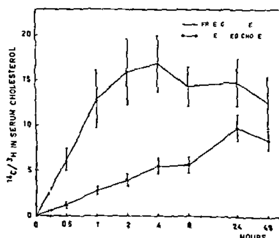


Fig The ratio C/^{3}H of both serum free and esterified cholesterol after simultaneous administration of DL mevalonate C and DL leucine-4 5 H intravenously to human subjects

Table II *Serum α ketoisocaproate concentration serum triglyceride pool and conversion of* DL *leucine-4 5 ^{3}H into serum triglycerides in man*

Case no	Serum α ketoisocaproic acid (mg 100 ml)	Serum triglyceride pool size (g[a])	Serum ^{3}H triglycerides (of dose at peak SA[b])
1	0 30	5 90	0 0873
2	0 59	7 03	0 0523
3	0 46	3 24	0 0302
4	0 66	3 06	0 0105
5	0 91	18 10	0 2170
6	0 64	5 23	0 0667
7	0 44	[illegible]28	0 0101
Mean	0 57	6 36	0 0677

[a] Plasma volume was assumed to be 4 5 % of body weight.
[b] SA = specific activity (dpm mg)

Table III *Turnover of serum esterified cholesterol in man*

Case no	Serum e t ri fied cholesterol (mg/100 ml)	Pool size (g[a])	Turnover of ^{14}C-cholesterol (/hour)	(mg/hour)	Turnover of ^{3}H cholesterol (/hour)	(m hour)
1	439	11 50	1 060	122 0	1 030	118 0
2	409	11 80	0 617	72 7	0 483	56 9
3	406	6 67	0 907	60 5	2 000	133 0
4	333	10 20	0 595	60 6	0 769	78 4
5	363	13 20	0 866	114 0	1 430	188 0
6	189	6 51	1 490	97 0	2 190	143 0
7	177	5 26	0 825	43 4	1 190	62 6
Mean	302	9 29	0 909	81 4	1 300	111 0
±S E			±0 115	+11 2	±0 240	±18 3

[a] Plasma volume was assumed to be 4 5 of body weight

more no significant amount of radioactivity was found in α ketoisocaproate isolated from 1 5 ml of serum 1/ h after administration of ^{3}H leucine Thus its turnover rate must have been very rapid or its specific activity was already initially very low

Incorporation of ^{3}H leucine to serum triglycerides

Because serum triglycerides are mostly synthetized in the liver the appearance of ^{3}H in this lipid class was measured in order to show whether ^{3}H leucine was converted to acetate in the liver Table II demonstrates that the fraction of ^{3}H leucine incorporated into serum triglycerides at the peak specific activity was approximately the same a that found in serum free cholesterol Further the larger the serum triglyceride pool the more ^{3}H *triglycerides were found in the circulation* ($r = 0\ 96$) No association was found between the radioactivity in serum triglycerides and serum free cholesterol

Turnover of serum esterified cholesterol

As presented in Fig 2 the ratio ^{14}C/^{3}H in serum sterified cholesterol increases constantly but more slowly than in free cholesterol up to 24 h Accordingly ^{3}H-cholesterol esters were released lightly faster than ^{14}C cholesterol esters into the irculation and in relation to ^{14}C cholesterol in higher concentrations than into free cholesterol This suggested that the turnover of ^{3}H esters could have been faster than that of ^{14}C esters Therefore the turnover data were calculated for *both ^{3}H and ^{14}C cholesterol esters It* will be seen from Table III that ^{3}H cholesterol esters actually turned over faster than ^{14}C esters in the two normocholesterolemic subjects and in three of five hypercholesterolemics The turnover rate of ^{3}H-cholesterol esters correlated to that of ^{14}C cholesterol esters ($r = 0\ 76$) However the calculated turnover rates as well as the fractional turnover rates were approximately the same in hypercholesterolemic and normocholesterolemic subjects and did not show any significant correlation to the radioactivities found in serum free cholesterol at the peak specific activity

DISCUSSION

The fraction of injected H leucine found in serum cholesterol in man was only about one hundredth of that of ^{14}C mevalonate The latter if taken up by the cell is however incorporated preferentially to cholesterol Leucine on the other hand could be incorporated into proteins or converted through several intermediary steps to HMG and further to ketone bodies and acetate (see Introduction) Thus the dilution of the radioactivity of leucine to its different metabolites before incorporation to cholesterol could have been markedly higher than that of ^{14}C mevalonate to its endogenous pool Furthermore ^{3}H leucine is first diluted into serum leucine (concentration 1–2 mg/100 ml (21)) followed by dilution into the intracellular pool the concentration of which is 30–50 times that in serum (21) Serum and tissue levels of mevalonic acid are not known

Since the liver at least in the rat has a low activity to metabolize leucine (mainly due to the small amount of leucine transaminase (11)) one may assume that the contribution of the *liver to* the cholesterol synthesis from leucine is negligible From the extrahepatic organs the muscle tissue catabolizes leucine readily (20) and has in addition a large mass being thus able to convert leucine at a high rate to HMG and possibly also to cholesterol It could be postulated that this cholesterol from muscle or from other extrahepatic organs may be the major source of serum *cholesterol known in man to originate largely*

from outside of the liver (3 22 23) The in vivo studies by Kabara have shown that leucine is converted to cholesterol with a relatively high specific activity in muscle of normal mice and in mice with muscular dystrophy (12) Our in vitro studies on rats demonstrated that cholesterol synthesis in muscle from leucine was higher than that from acetate or even from mevalonate Further the incorporation of this amino acid into serum cholesterol was reduced markedly less than that of acetate in rats whose hepatic cholesterol synthesis from acetate and HMG was blocked by cholesterol feeding (18)

The relatively high conversion of leucine to serum triglycerides in subjects of the present study indicates that this amino acid (or its metabolites e g α ketoisocaproate) was actually metabolized by the liver not only to HMG but also further to acetate and fatty acids Thus the hepatic H-cholesterol may have been synthetized from ^{3}H HMG formed either directly during leucine catabolism or from ^{3}H acetate after the acetate pool was labelled by the breakdown of ^{3}H HMG The extensive dilution of ^{3}H leucine and the apparent hepatic synthesis of ^{3}H cholesterol make it difficult to evaluate the quantitative role of leucine as the precursor of serum cholesterol in man and the contribution of extra hepatic tissues to synthesis of serum cholesterol from leucine

Although the amount of free ^{3}H cholesterol in serum and the turnover of serum esterified ^{3}H cholesterol after ^{3}H leucine was generally in accord with the respective ^{14}C values from ^{14}C mevalonate some differences were found The faster appearance of free ^{3}H-cholesterol in serum as compared to that of ^{14}C-cholesterol suggested that the intracellular localization of cholesterol synthesis from the two precursors and thus the release of resulting free ^{3}H and ^{14}C cholesterol into the circulation could have been partially different More probably however the uptake and the subsequent conversion to cholesterol of leucine were faster than of mevalonate in one or several tissues This early release seems not to be specific for leucine-cholesterol because by employing the simultaneous administration of ^{3}H mevalonate and ^{14}C acetate (15) ^{14}C-cholesterol appeared faster and remained longer in serum than ^{3}H-cholesterol resulting in a peak in the ^{3}H/^{14}C time curve (16) just as the ratio ^{14}C/^{3}H did in the present study The fast labelling of serum free ^{3}H-cholesterol could have resulted in a faster esterification and higher turnover of ^{3}H cholesterol as compared to ^{14}C cholesterol However the radioactivity found in serum free cholesterol at the peak specific activity did not correlate to the turnover of the serum esterified cholesterol Although the esterification is known to take place slowly also in plasma (7) Kabara (12 13) observed that the specific activity of esterified tissue cholesterol was higher than that of free cholesterol within 15 min after administration to mice of either ^{14}C leucine or H acetate Cholesterol formed from these precursors had however a higher esterification than that formed from ^{3}H mevalonate This could explain the fast appearance of esterified ^{3}H cholesterol in serum in the present study

The amount of ^{14}C acetate or ^{14}C mevalonate appearing in plasma free cholesterol has been pointed out to correlate to the size of the plasma free cholesterol pool (10 19) The results of the studies on ^{14}C cholesterol confirmed this finding in the present study while the incorporation of leucine seemed to be less dependent on the pool size of serum free cholesterol Furthermore Hennes et al (10) but not Nestel and Monger (19) found the reduced rate of fall in the specific activity of plasma free cholesterol during the first day in hypercholesterolemic subjects a finding confirmed in the present study for ^{14}C but not for ^{3}H cholesterol The significance of these findings remain uncertain The relatively high amount of radioactivity appearing in serum free ^{14}C cholesterol pool of hypercholesterolemic subjects of the present study is hardly an indicator of the magnitude of endogenous cholesterol synthesis mainly because the sterol balance which in the steady state equals the endogenous cholesterol synthesis appears to be normal in hypercholesterolemic subjects (17) The lack of correlation between the portion of the label transferred into the serum free cholesterol pool at the peak specific activity and the turnover of serum esterified cholesterol suggests that the former is not either primarily dependent on the esterification of serum free cholesterol Assuming that the equilibration of newly formed serum free cholesterol to other free cholesterol pools proceeds at a constant rate independently of the pool size the amount of label found in plasma free cholesterol

is high in hypercholesterolemia while the rate of fall of the specific activity is low

The fractional turnover rate of cholesterol esters that tended to be higher for ^3H than for ^{14}C cholesterol was somewhat lower than reported by Nestel and Monger (19) Even then however the daily average turnovers of ^3H cholesterol and ^{14}C cholesterol were about 2 7 and 2 0 g respectively In the steady state this amount must also be removed The removal probably involves mainly the hydrolysis of the esters back to free cholesterol (8) at least it cannot be exclusively due to transfer of the esters into faeces by the liver and intestine because the daily faecal excretion of cholesterol and bile acids usually appears to be less than 1 g (17)

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REFERENCES

1 Bloch K Some aspects of the metabolism of leucine and valine J biol Chem 155 255 1944
2 Carlson L A Determination of serum triglycerides J Atheroscler Res 3 334 1963
3 Cox G E Taylor C B Patton D Davis C & Blandin N Origin of plasma cholesterol in man *Arch Path 76 60 1963*
4 Dancis J Levitz M & Westall R G Maple syrup urine disease branched chain keto aciduria Pediatrics 25 71 1960
5 DeSchepper P Parmentier G & Vanderhaeghe H A study of the α keto acids in blood Biochim biophys Acta (Amst) 28 507 1958
6 Dietschy J M & Siperstein M D Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat J Lipid Res 8 97 1967
7 Glomset J A The mechanism of the plasma cholesterol esterification reaction plasma fatty acid transferase Biochim biophys Acta (Amst) 65 128 1962
8 Goodman De W S Cholesterol ester metabolism Physiol Rev 45 747 1965
9 Hansen, P W & Dam H Paper chromatography and colorimetric determination of free and esterified cholesterol in very small amounts of blood Acta chem scand 11 1658 1957
10 Hennes A R Moore M Z & Masters Y F Studies of cholesterol metabolism with C acetate in diabetic patients and in patients with hypercholesterolemia Metabolism 11 925 1962
11 Ichihara A & Koyama, E Transaminase of branched chain amino acids J Biochem 59 160 1966
12 Kabara J J Brain cholesterol VI The effect of hereditary dystrophia muscularis on (C)leucine and (2 H)acetate incorporation Tex Rep Biol Med 22 134 1964
13 — Brain cholesterol VII The effect of hereditary dystrophia muscularis on (2 C)mevalonic acid and (2 H)acetate incorporation Tex Rep Biol Med 22 143 1964
14 *Meister A Valine* isoleucine and leucine In Biochemistry of amino acids vol II p 729 Academic Press New York 1965
15 Miettinen T A Effect of dietary cholesterol and cholic acid on cholesterol synthesis in rat and man Biochem Pharmacol 4 68 1967
16 — Unpublished results
17 Miettinen T A Pelkonen R Nikkila E A & Heinonen O Low excretion of fecal bile acids in a family with hypercholesterolemia Acta med scand 182 645 1967
18 Miettinen T A & Penttila I M Incorporation of H leucine C acetate and H mevalonate to serum and tissue cholesterol in the rat Scand J clin Lab Invest Suppl 95 92 1967
19 Nestel P J & Monger E A Turnover of plasma esterified cholesterol in normocholesterolemic and hypercholesterolemic subjects and its relation to body build J clin Invest 46 967 1967
20 Penttila I M Effect of insulin chlorpropamide and tolbutamide on the metabolism of branched chain amino acids Ann Med exp Fenn Suppl 11 1 1966
21 *Roberts E & Simonsen D G Free amino acids in* animal tissues In Amino acid pools (ed J T Holden) p 284 Elsevier Amsterdam 196
22 Taylor C B Mickelson D Anderson J A & Forman D T Human serum cholesterol synthesis measured with the deuterium labels Arch Path 81 213 1966
23 Wilson J D & Lindsay C A Jr Studies on the influence of dietary cholesterol on cholesterol metabolism in the isotopic steady state in man J clin Invest 44 1805 1965
24 Zilversmit D B The design and analysis of isotope experiments Amer J Med 29 832 1960

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KÖHLMEIER DEGOS DISEASE (MALIGNANT ATROPHIC PAPULOSIS)

Report of the First Scandinavian Case

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Abst act A report is given of the first Scandinavian case of malignant atrophic papulosis (Kohlmeier Degos disease) in a man aged 31 The patient presented the typical morphological changes in the skin and intestine This case is the first in which similar vascular lesions have been demonstrated in the lungs liver and pancreas

In 1941 Kohlmeier (4) described a remarkable cutaneo-intestinal syndrome in a 21 year old man and assumed the findings to be manifestations of thrombo-angiitis obliterans In the following year Degos et al (2) reported a similar case and thought the syndrome to be a well defined entity which they first called papulo squamous dermatitis but afterwards malignant atrophic papulosis to indicate the poor prognosis of the condition

A further 19 cases have since been published The literature has been reviewed by Sidi et al (7) and by Strole et al (8)

The case reported below is the first known in stance of this rare disease in Scandinavia It is also the first case in which typical vascular lesions of Kohlmeier–Degos disease have ever been found in the lungs liver and pancreas The diagnosis was made at histological examination post mortem and historical data particularly those relevant to the evolution of the cutaneous lesions were far from complete

CASE REPORT

A man aged 30 had mild pneumon a in 196 and in 1963 In the summer of 1965 he had a rash on the trunk and thighs Later (after the patient had d ed) the pa tient s w fe described these les ons as red patches which afterwards became pale f rst centrally and then disappea ed That year he also had diffuse epigastric pa n but roentgenography revealed noth ng remarkable In August 1966 the symptoms ncreased with frequent vomiting and considerable loss of body we ght (0 kg within two months) He was admitted to his local hospital for in vestigation A few days after admission he had per forating ulcer in the anterior part of the stomach for which he was operated upon in O tober 1966 Careful abdominal palpation and examination revealed nothing remarkable apart from slight enlargement of the spleen Chest X ray after the operation showed diffuse coalescent densities on both sides (no abnormalities found at chest X ray in 1964) He was transferred to the Chest Clinic Boden for further investigation He had fever (38–39 C) for a week and on December 4 1966 an acute attack of abdominal pain suggesting perforation Surgical explora tion revealed perforation of the ileum with cons derable local changes for which the intestine was resected and anastomosed end to-end He was then transferred to the department for intense therapy

On admiss on to that department a careful inquiry was again made into the patient s h story this time including also a search for signs of intoxication and allergy but revealed nothing of interest The patient was very cachectic but otherwise in a good general condition Chest X ray showed further progression of the densit es in both lungs The dens ties were partly coalescent but here and there they were rounded and well defined and appeared mal gnant ESR 30 mm/h Hb 11 1 g/100 ml R B C 4 mill Hct 38% platelet count 72,000 W B C 8300 with 76% neutrophils Serum electrolytes normal Serum protein 4 4 g/100 ml Electrophoresis albumin 44 alpha -globul n 10 8% alpha$_2$ globulin 14 9% beta glob-ul n 15 9% and gamma globulin 14 1% Plasma creati nine 0 7 mg 100 ml Urine salts specific gravity and osmolarity normal Urine sediment -3 red blood cells 5–10 white blood cells only few bacteria Sternal punc ture markedly increased myelopoesis with shift to the left and dominated by myelocytes Increased number of mature plasma cells Reticulum markedly increased In creased alkaline phosphatase activity of neutrophil

Culture of throat swabs bronch al secretion urine and blood was repeatedly negative Repeated examination for mycobacteria with microscopic examination culture and guinea pig inoculation negative Culture and agglutination for pseudotuberculos s negative Antistreptolys n titre 70 units per ml antistaphylolysin titre 0 36 unit per ml No agglutination of Salmonella O-antigen H antigen Bru cella abortus or Tularense Bronchoscopy with b opsy and

cytological examination revealed nothing remarkable

Because of his cachectic condition the patient was given adequate nutrition parenterally Tuberculosis was stron ly suspe ted and treatment with PAS INH and streptomycin was started on December 7 Fever persisted (38–39 C) and cardiac frequency was high 120–130 ECG normal except for tachycardia On December 10 the body t mperature rose still more (41 C) and a chest X ray showed progression of the changes besides which the liver was palpably enlarged PAS was therefore with drawn On December 14 he again had peritonitis and was again subjected to surgical exploration No p rfora tion was found but the small bowel showed marked chan es with fibrin-coated lesions and a considerable amount of whitish round infiltrates with palpable thicken ing of the serosa A small part of the ileum was excised Th patient tolerated the operation fairly well Microscopical examination of the material obtained at the operations yielded no information of diagnostic value

The operati e specimens were rou hly 10 cm long seg ments of the ileum with diffu e thickening of the wall and numerous pale gray to yellow irregular plaques on the serosal surface Centrally there was a perforation, the size of a peppercorn and an adjacent circular stric ture The mu osal fold were exag erated

Microscopically the perforation was lined by thick fibrinous necrotic masses and bordered by granulation tissue with abundant inflammatory cells mainly granu locy es Adjacent submucosa was oedematous with dilated ve els and mild fibrosis The middle sized arteries showed subendothelial fibrosis with marked narrowing of the r lumina Some areas of the serosal surface were covered by a thin fibrin coat with an admixture of granulocytes and single fungi of the species *Candida albicans*

The pathological diagnosis was infarction of the ileum of obscure origin with perforation and peritonitis In retrospect, the lesions were identical with those described and illustrated under necropsy findings

One week after the operation the patient had chills and intense parenteral treatment with penicillin (4 mill units per hour) was tried with simultaneous steroid therapy to suppress the pulmonary reaction For a few days th fever disappeared, but tachycardia persisted On December 28 he had symptoms of a new perforation Explorative surgery revealed a palm sized perforation of the stomach and severe peritonitis The lesion was closed with a fairly satisfactory result The patient made a good recovery but had high grade fever and tachycardia At operation tracheotomy was done and even after the opera tion the patient could not mana e without a respirator As before new laboratory and bacteriological tests proved negative The serum bilirubin and transaminases gradu ally increased and the patient developed a bleeding tendency with an Ivy bleeding time of more than 36 min The plastic clotting time was normal Fibrinolytic activity with fibrinolysis 0 2 mg/ml/h and fibrinogenolysis 0 31 mg/ml/h, fibrinogen 0 15 g/100 ml He was given transfusions of fresh blood and EACA, which resulted in normalisation of these values and his bleeding time On January 1 1967 treatment with erythromycin was tried because of a period of high grade fever and further progression of the pulmonary chan es The response was favourable the fever disappeared and for the first time the pulse rate became normal and the patient s general condition improved Within a few days the pulmonary changes regressed considerably After four days the serum bilirubin b gan to rise rapidly the liver increased further in size tachycardia returned and body temperature rose Erythromycin was withdrawn and other antibiotics were tried but without effect On January 9 he was treated with hypothermia with fair success until January 13 when the patient went into irreversible shock and died on January 16

Necropsy findings

Complete necropsy including the brain, was performed at Centrallasarettet Boden The external examination re vealed jaundice and generalised petechial cutaneous haemorrhages The chief finding was fibrinopurulent pe ritonitis with widespread adhesions and a right-sided sub diaphragmatic abscess *The gastrointestinal tract was di* lated and the content heavily bloodstained The intestinal serosa was covered by thick patches of pus and the intestinal wall was o dematous The mucosa of the stomach and small intestine was thickened oedematous and showed widespread haemorrhages The middle of the dorsal wall of the stomach had a 1 5 × 1 5 cm perfora tion coalesced with the left liver lobe

The mesenteric lymph nodes were enlarged The mesenteric vessels were dissected but no nodules thrombi or other gross changes were found

The liver showed well defined haemorrhagic infarcts No gross changes were seen in the large trunks of the hepatic artery or portal vein

Other autopsy findings were scattered small necroses in pancreas tracheobronchitis and multiple haemorrhagic pulmonary infarctions up to the size of almonds No thrombi were seen in the main branches of the pulmonary artery

Microscopical study of the operative sp cimens and specimens obtained at autopsy showed largely uniform pathological appearance of affected vessels in the gastro intestinal tract liver pancreas and lungs The typical *picture consisted of intravascular endothelial proliferation* and progressive occlusive fibrosis in small and medium sized arteries (Figs 1–4) with or without secondary thrombosis and consequent ischaemic infarction

The lesions in the gastrointestinal tract, from the stomach through colon, were of uniform appearance and involved all the layers of the intestinal wall The submucosa was affected most with severe congestion and oedema The blood and lymph vessels were dilated and often surrounded by fibrous tissue The arterial walls were irregularly thickened hyalinised and encircled by a few lymphocytes The disorganisation was due to an eccentric subendothelial fibrosis with preserved ela tica interna leading to o clusion of small vessels and to re duction in the size of the lumina of larger vessels Thrombi of various ages and sizes were adherent to the vessel walls Veins were also affected though to a lesser degree Occasionally though rarely a few areas of active vasculitis were recognized with involvement of the vessel wall by lymphocytes histiocytes and some neutrophils



Fig. 1. Submucosal artery in ileum (operative specimen) with marked subendothelial fibrosis. Haematoxylin-eosin ×100.

Fig. 2. Same section as in Fig. 1. Internal elastic lamella intact. van Gieson-elastica ×100.

Fig. 3. Submucosal artery in ileum. Marked subendothelial fibrosis and mild lymphocyte reaction in surrounding stroma. Haematoxylin-eosin ×100.

Fig. 4. Same section as in Fig. 3. Internal elastic lamella intact. van Gieson-elastica. ×100.



Fig 5 Pulmonary infarction with two small arteries almost occluded by loose subendothelial fibrosis van Gieson elastica × 85

The mucosa showed patches of necrosis with ulcerations and splitting of the muscularis mucosae In severely affected areas and around the large perforation in the stomach all layers of the wall were infarcted and showed signs of superimposed infection with abundant inflammatory cells mainly neutrophilic leucocytes and some fungi of the species *Candida albicans*

Other segments of the intestine showed vascular changes also in muscularis propria and some fibrous tissue was deposited in both muscle layers There was abundant fibrinopurulent exudate on the serosal surface and in the subserosal connective tissue

The mesenteric lymph nodes showed non specific inflammation and no vascular changes

Lesions identical with those in the gastrointestinal tract were seen in small blood vessels in the lungs (Fig 5) liver and pancreas (Fig 6) The liver and pancreas showed widespread haemorrhagic necro es with well defined leucocytic margins The liver surface was covered with thick patchy layers of fibrin containing numerous granulocytes Also the infarcted pulmonary parenchyma showed abundant granulocytes and the adjacent lung tissue was covered by haemorrhagic pneumonia without any histologically demonstrable microorganisms The visceral pleura was fibrously thickened and its surface was partly covered by fibrin swarming with leucocytes

No morphological changes were found in the brain

DISCUSSION

Kohlmeier Degos disease papulosis atrophicans maligna is a remarkable systemic disorder of the vessels It is characterised by practically pathognomonic skin lesions The gastrointestinal tract is the most often affected of the organ systems involved and the outcome is generally fatal owing to multiple intestinal infarctions with perforations and consequent fulminating peritonitis The cause of the disorder is obscure and no effective treatment is yet available Clinically the disease is readily distinguished from polyarteritis nodosa and thrombo angiitis obliterans and from a pathological point of view it cannot be regarded as a manifestation of these diseases

The cases on record were seen in 17 men and two women The commonest age of onset is 15 to 25 years The disorder is usually fatal within one year of the appearance of the skin lesions

The initial skin lesions which are scattered over the trunk neck and proximal portions of the limbs develop slowly The lesions are asympto



Fig 6 Eccentric subendothelial f brosis of medium sized artery in pancreas Adjacent parenchyma is partly necroti and shows scattered round cells van Gieson-elastica × 85

matic pink to red rounded or oval papules Within a few days they become umbilicated and there appears a porcelain white atrophic centre encircled by a slightly elevated erythematous tele angiectatic border Some weeks later the lesions begin to disappear and leave behind atrophic white spots but lesions in different stages of de velopment can always be found

Histopathologically the picture of the skin eruptions is that of necrosis in dermis and sub-cutaneous tissue with underlying oedema and a non specific inflammatory reaction which is fol lowed by sclerotic atrophy in the dermis and atrophy and hyperkeratosis of the epithelium. These lesions are the result of focal changes of small and medium sized arteries in the subepi dermal tissues with progressive subendothelial oc clusive fibrosis with consequent ischaemic in farcts Veins and lymphatics are affected later by peripheral fibrosis. The skin and small bowel are the commonest sites of the spotty vascular changes and the abdominal symptoms are as a rule ushered in by the skin lesions The onset of the abdominal symptoms generally marks the beginning of steady deterioration with a fatal issue As long as the bowel is only slightly affected the patient generally feels well except for mild abdominal symptoms and fair loss of weight Progression of the in testinal lesions is accompanied by ileus nausea, haematemesis and melena but an acute attack of intense epigastric pain may be a sign of the initial stage of the abdominal syndrome

Morphologically the intestinal lesions do not differ from the cutaneous lesions but the vascu litis thrombosis and adjacent inflammation are often more prominent in the intestine than in the skin

Contrary to what was supposed on the basis of the first cases published the disease is not con fined to the skin and/or intestines Thus identical focal vascular changes damaging the tissue have been described in the brain kidney heart eyes mesentery and bladder (1 3 5 6 7 8 9) This paper is the first to report similar vascular changes in the lungs liver and pancreas Such lesions may perhaps be found also in other organs and possibly early enough to provide a clue to their aetiology

REFERENCES

1 Culicchia C F., Gol A & Erickson E E Diffuse central nervous system involvement in papulosis atrophicans maligna Neurology (Minneap) 12 503 1962.

2 Degos, R., Delort J & Tricot R Dermatite papulo-squameuse atrophiante Bull. Soc franç Derm Syph 49 148 194_

3 Gever S G Freeman R G & Knox, J M Degos disease (papulosis atrophicans maligna) report of case with degenerative disease of the central nervous system S.h med J (Bgham., Ala) 55 56 196_

4 Kohlmeier W Multiple Hautnekrosen bei Trombo-angiitis obliterans Arch Derm Syph (Berl) 181 783 1941

5 Naylor D., Mullins J F & Gilmore J F Papulosis atrophicans maligna (Degos disease) report of first United States case and review of literature Arch Derm 81 189 1960

6 Nomland R & Layton, J M Malignant papulosis with atrophy (Degos) fatal cutaneointestinal syn drome Arch Derm 81 181 1960

7 Sidi, E., Reinberg, A Spinasse J B & Hincky M Lethal cutaneous and gastro intestinal arteriolar throm bosis *(malignant atrophying papulosis of Degos)* J.A.M.A 174 1170 1960

8 Strole W E, Jr., Clark W H Jr & Isselbacher K. J Progressive arterial occlusive disease (Kohlmeier Degos) A frequently fatal cutaneosystemic disorder New Engl J Med _76 195 1967

9 Winkelmann, R. K., Howard F M Jr Perry H O & Muller R H Malignant papulosis of skin and cerebrum A syndrome of vascular thrombosis Arch Derm 87 54 1963

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THE PROPHYLACTIC ANTIARRHYTHMIC EFFECT OF QUINIDINE IN MYOCARDIAL INFARCTION

A Controlled Clinical Trial

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Abstract A controlled study has been carried out to examine the possible prophylactic antiarrhythmic effect of quinidine sulphate 0.2 g t.i.d. in acute myocardial infarction. No difference was found in the occurrence of the various types of arrhythmias apart from extrasystoles, which were significantly less frequent in the treated than in the non treated group. An analysis of the causes of death revealed no difference in sudden and unexpected deaths between the two groups. There was rather a vague impression that quinidine might have contributed to dangerous complications and death in a few patients. The possible reasons for these findings are discussed in relation to the observations from the literature.

Rates of mortality and statements of death mechanisms in acute myocardial infarction vary considerably in the different series published. The mechanisms of death are usually put down as arrhythmias, cardiac failure and cardiogenic shock. In many cases it is difficult to decide what is the main and what is the secondary cause. Mower et al (11) attribute 50% of the deaths to "rhythm disturbances", 30–35% to heart failure and the rest to cardiogenic shock. Hellerstein and Turrel (6) claim that 40 to 50% of the deaths due to myocardial infarction are caused by "electrical failure" while the rest are due to "mechanical failure". In other words about one half of all deaths due to myocardial infarction seem to occur from disturbances of normal impulse formation and conduction. Many of these cases suffer a "sudden and unexpected death" and undoubtedly some of these patients may have "hearts too good to die". If a further reduction in mortality is to be obtained it seems rational to concentrate on this category of patients. Much less is to be expected from the treatment of patients with mechanical failure due to severe myocardial damage caused by the infarctions.

The prophylactic use of antiarrhythmics in acute myocardial infarction has been scarcely dealt with in the literature. The results of the few properly conducted examinations vary so much that it is difficult to draw any definite conclusions.

Cutts and Rapoport (4) giving up to 0.8 g quinidine t.i.d. found no effect upon mortality and frequency of arrhythmias, heart failure or shock.

Boone and Pappas (2) found a highly significant improvement of the mortality rate in a group treated with quinidine compared with a control group. Their work, however, is retrospective and the groups are heterogenous (for instance with respect to anticoagulant therapy) and numerically very different.

Scherf (13) proposes that all patients with acute myocardial infarctions routinely should have quinidine except when contraindications exist.

Berg (1) and Reynell (12) found no effect on mortality rate when giving quinidine and procainamide respectively. The effect on the arrhythmias was uncertain and inconclusive.

Hvidt et al. (9) report no effect of quinidine sulphate 0.2 g t.i.d. on mortality rate. Nor do they find any prophylactic action against arrhythmias. They also state that quinidine may be harmful on account of the depressive action on impulse formation and conduction. Their quinidine group and control group however are from different periods of time.

Table I *Composition of material*

Age distribution	Quinidine group (94) ♀	Quinidine group (94) ♂	Control group (107) ♀	Control group (107) ♂
<50	0	6	1	12
50–59	3	14	4	18
60–69	9	22	6	33
70–79	15	18	10	16
>80	5	2	0	7
Total	32	62	21	86
Average age	71 5	63 4	63 9	63 3
Range	51–84	42–81	46–79	36–89

With reference to the discrepancy between the conclusions in the different series published the prophylactic antiarrhythmic effect of quinidine has been studied in a controlled clinical trial

MATERIAL AND METHODS

All patients with acute myocardial infarction and a case history shorter than 48 hours before admission to the hospital were included during the period from September 1 1964 to August 31 1966 All the patients who died within the first 12 hours were excluded from the material Ninety four patients arriving on odd days of the month were given quinidine sulphate 0 2 g three times a day One hundred and seven patients arriving on even days as controls No patient has been included more than once

The plan was to try to answer the following questions

1 Does quinidine 0 2 g three times daily provide any prophylactic effect against (*a*) Sudden cardiac death? (*b*) Arrhythmias (some of which might be potentially fatal)?
2 Are signs of myocardial depression recorded due to the above mentioned dose of quinidine?
3 Does quinidine 0 2 g three times daily cause dangerous or fatal arrhythmias or blocks?
4 How often do side-effects necessitate withdrawal of quinidine in an unselected group of patients with acute myocardial infarction?

A relatively small dose of quinidine was chosen because larger doses routinely might produce more toxic reactions

Cases with atypical ECG (about one third of the material) were included provided that the case history and biochemical findings made the diagnosis certain

The patients were examined at least twice daily Special attention was paid to auscultation of the heart notes being made about gallop rhythm friction rubs arrhythmias signs of cardiac failure and thromboembolism We were especially aware of side-effects of quinidine

Table II *Complicating diseases*

	Quinidine group ♀	Quinidine group ♂	Control group ♀	Control group ♂
Hypertension	14	22	10	28
Diabetes	9	5	3	5
Previous apoplexia	0	3	0	6
Rheumatic heart disease	6	1	2	0
Previous angina pectoris	19	29	13	34
Previous infarction	8	8	4	15
At least one complicating disease (except angina pectoris *and/or infarction)*	49		40	
Two or more complicating diseases	12		5	
Shock on admission	20		31	
Median SGO T value *(Sigma units)*	118	180	150	156

Table III *Localisation of infarctions*

	Quinidine group ♀	Quinidine group ♂	Control group ♀	Control group ♂
Anterior	9	25	10	22
Posterior	13	15	6	33
Anterior + posterior	0	0	0	2
Atypical ECG	10	22	5	29

Table IV *Digitalis treatment started during hospitalisation*

Indications	Quinidine group	Control group
Left ventricular failure	20 (2†)	25 (4†)
Right ventricular failure	0	0
Combined failure	5 (1†)	4 (1†)
Arrhythmias	5 (0†)	4 (1†)
Arrhythmias + failure	4 (1†)	1 (0†)
Shock	2 (1†)	5 (3†)
Shock + failure	4 (1†)	5 (5†)
Cardiomegaly (without obvious failure)	0	2 (0†)
Total	40 (6†)	46 (14†)
Previously treated with digitalis	14 (4†)	13 (2†)
Total number of cases with previous or present digitalis treatment	54 (10†)	56 (16†)
Left hospital on digitalis treatment	44	43

Table V *Arrhythmias at hospital (arrhythmias during first 24 h not included)*

Type of arrhythmia	Quinidine group	Control group
Extrasystoles	*49*	*58*
Atrial fibrillation	13	15
Atrial flutter	4	3
Sinus tachycardia	19	17
Sinus bradycardia	9	13
Nodal rhythm	3	4
Nodal tachycardia	1	1
AV block (Grade I–III)	10	12
Ventricular tachycardia	2	2
Ventricular fibrillation	*1*	*1*
Patients with one or more of the above mentioned disturbances of rhythm and/or conduction	69	79
Patients without any recorded arrhythmia or conduction disturbance	25	28

Table VI *Survey of death rates*

	Quinidine group	Control group
Arrived in hospital within 48 h from onset of symptoms	*103 (67 ♂)*	*11[illegible] (91 ♂)*
Died during the first 12 h in hospital	9 (5 ♂)	5 (5 ♂)
Left hospital alive	85 (58 ♂)	89 (72 ♂)
Total mortality	$\frac{18}{103}$ 100 = *17 5*	$\frac{23}{11[illegible]}$ 100 = *20 5*
Mortality during observation period	$\frac{9}{94}$ *100 = 9 6*	$\frac{18}{107}$ *100 = 16 8*
Male mortality during observation period	$\frac{4}{62}$ 100 = *6 5*	$\frac{14}{86}$ 100 = *16 3*
Female mortality during observation period	$\frac{5}{32}$ 100 = *15 6*	$\frac{4}{21}$ 100 = *19 0*

All patients were treated with anticoagulants (phenyl *indanedione). An attempt was made to keep the thrombo* test value between 10 and 20%. They were early out of bed i e when temperature signs of shock or heart failure did not contraindicate (in most patients within one week). Patients with uncomplicated infarctions usually left the hospital within three weeks

The two groups are fairly comparable as a whole with regard to age distribution complicating diseases presence of shock and values of SGO T (Tables I and II) Furthermore no significant difference was observed between the groups concerning the localisation of the infarctions or digitalis treatment during hospitalization (Table III and IV)

Table VII *Survey of deaths in quinidine group*

I = Previous infarction A p = angina pectoris A = anterior infarction P = posterior infarction Atyp = atypical ECG

Initials	Age	Sex	Complicating heart disease	Type of infarct	Quinidine side-effects	Causes of death: Primary	Causes of death: Secondary	Time of death (d)
J A	78	♀	Aortic stenosis Aortic insuff I 2 years ago A p 5 y	A		Shock		1½
A H	72	♀	A p 5 y	P		Mors subita		4
O H	66	♂	I 1½ y	A	Asthenia Anorexia	Shock (re inf)	Heart failure	21
K J	79	♀	Aortic stenosis Mitral insuff I 1 year ago A.p 4 y	Atyp		Ventric fibrill		25
T L	65	♂	A p 6 y	P		Mors subita	Pre shock	8
G R	74	♂		A		Mors subita	Heart failure	19
A U	64	♂	I 2 years ago A p 2 y	Atyp		Shock	Heart failure Nodal tachyc	5
L Ö	81	♀		A	AV block (grade 3)	Shock	Heart failure	2
A Å	76	♀	A p 2 y	P	AV block (grade 3)	Shock	Heart failure Embolus of left fem artery	5

Five ♀ average age 77 [illegible] y
Four ♂ average age 67 3 y

Table I *The calculated number of human Taenia infections in millions*

Geographical area	Taenia solium	Taenia saginata
North America	< 0 1	21 5
Middle and South America	< 0 1	1 3
Africa	0 5	0 2
Europe without USSR	< 0 1	3 9
USSR in Europe	0 5	1 3
USSR in Asia	0 3	—
Asia excluding USSR	1 2	< 0 1
Oceania	< 0 1	< 0 1

CASE REPORT

Mechanic aged 56 married right handed Admitted December 1965 Nearly seven months history of periods lasting about ten seconds of vertigo feeling of unreality slight nausea and inadequate speech The symptoms appeared from two to three times a week Moreover the patient felt that his memory had deteriorated Past history was noncontributory Physical examination showed nothing remarkable Laboratory findings were normal but ESR was 20 mm EEG showed a moderate focal abnormality partly episodical but otherwise unspecific maximum in the left frontotemporal area, like temporal lobe epilepsy Conventional films of the skull showed a calcified corpus pineale and small calcifications of the ependyma and pneumoencephalography showed a downward convexity in the roof of the left lateral ventricle An angiography in the left common carotid artery (completed in the right side) revealed a parasagittally situated poorly vascularized expansive lesion in the middle and back of the parietal area which was the size of a hen's egg The patient was referred to the Neurosurgical Clinic at Karolinska Sjukhuset where in March 1966 an extracerebral irregular and in some places lobulated cyst was removed It contained a green whitish milky fluid The cyst was situated in the middle third of the venous sinus Histologic diagnosis showed a colloid cyst without malignant features In the postoperative course the patient had epileptic seizures of a generalized character which were alleviated by the administration of antiepileptic drugs The patient s only complaint was an unpleasant olfactory sensation with almost complete anosmia An initial hemiparesis of the right side regressed completely A feeling of weakness in the right arm and leg could not be verified objectively Laboratory studies were normal

The patient lived at home for three months and was given antiepileptics He suffered from one or two recurrent epileptic seizures every month he was unconscious for five minutes but without involuntary urination

In the middle of July 1966 a right sided hemiparesis developed in the course of four days On admission the patient could walk by himself There was weakness in his right arm and leg, and slightly increased tendon reflexes on this side Lumbar puncture showed normal cerebrospinal fluid pressure and a normal Queckenstedt s sign Lumbar fluid was clear colourless without increased cell count and protein 42 mg the Mastix reaction was normal The hemiparesis progressed and therefore in August 1966 the patient was again referred to the Neurosurgical Clinic at Karolinska Sjukhuset The EEG then showed distinct deterioration compared with his condition in April 1966 An angiography in the left common carotid artery revealed a large nonvascularized area in the left parietal region At operation no recidivation of the former cyst was observed but numerous small cysts containing clear fluid bulged from the subarachnoid space From the cortical surface towards the dura and the falx a large number of arachnitic chords were seen, with a yellowish coating The vasculature of the cortical surface was increased in the same way as in a chronic inflammatory state Some yellow whitish rounded corpuscles each as large as a grain of rice were found in the arachnoidea toward the falx. Histologic diagnosis of the extirpated tissue revealed a cysticercosis In the postoperative course paresis of the right leg diminished while that of the right arm was almost complete Owing to the diagnosis of cysticercosis repeated examinations for ova in the feces were made but with no result Radiologic investigations of the lungs and the muscles of the femora did not show any abnormal findings The total eosinophilic leucocyte count was normal as were subsequently repeated differential counts Since his discharge in November 1966 the patient has lived at home taking antiepileptics and going in for physical training Paresis has regressed to some extent and a year after discharge the patient is able to take long walks without support He can raise his arm up to the level of his shoulder The olfactory sensation is unchanged The patient has suffered from five minor epileptic seizures at night These were of short duration and without involuntary urination EEG is essentially unchanged

The patient was brought up on a farm where among other animals pigs were bred From 1930 to 1950 he worked as a groom and then as a mechanic However each year from about 1936 to 1955 he has bred four domestic pigs for slaughtering and use in the household This has been done without meat inspection He has not travelled abroad which could have involved a risk of exposure to taenia There is no history of disease caused by worms except an oxyuriasis infection in childhood

DISCUSSION

Extensive reports from different parts of the world show that there is relative agreement in the age distribution of patients with cysticercosis cerebri The maximum age when the affection is contracted is between 20 and 40 and up to 50 years Initially the parasites cause only minor disturbances and the patient can live for years with mild symptoms even with numerous cystic lesions in both hemispheres Most often the cysticerci are found in the subarachnoid space in the basal nuclei in the ventricles and sometimes in

an intracerebral location According to the symptomatolo_gy different classifications have been introduced (8 9)

Throughout the most frequent form seems to be the convulsive type of symptoms with initial symptoms of focal origin irrespective of whether their form is Jacksonian or temporal lobe epileptic In a large percentage of these cases the EEG show an active cerebral focus The symptoms in the same patient vary widely during the course of the disease with mental disturbances and generalized epilepsy At a multiple localisation there may be signs of increased intracranial pressure and cerebral edema The cerebrospinal fluid may show eosinophilia, hypercytosis slightly increased protein content with qualitative changes such as a raised gammaglobulin fraction A positive reaction of complement fixation in a satisfactory titer for diagnosis exists in about 70

The hypertensive type of symptoms with headache vomiting and papilloedema may mainly depend not only on a basal localization of the parasite with basal chronic leptomeningitis as a consequence but also on engagement of the fourth ventricle or fossa posterior Especially when localization is in the fourth ventricle there will be intense symptoms owing to a total or subtotal blockade of the circulation of the cerebrospinal fluid from membranous formations which also surround the parasite

The diagnosis is much facilitated if the patient has subcutaneous noduli accessible to biopsy or muscular or intracranial calcifications visualized by roentgenography Arseni and Samitca (1) found that in about 90 of 65 cases of cysticercosis cerebri the cysticerci were localized solely in the brain Only 10 of the cases with radiologically demonstrable muscular calcifications had intracranial calcifications which by no means exclude cerebral cysticercosis There are few reports in the literature dealing only with the roentgenologic aspects of the condition In an investigation of this kind Dorfsman (3) showed the presence of intracranial calcifications in four out of 126 cases verified at operation or post mortem On the other hand the percentage of intracranial calcifications in clinically diagnosed cases of cysticercosis cerebri is said to be as high a 15

The classification of cysticercosis cerebri proposed by Stepien (9) and based on the clinical course in cases resembling 1 tumor 2 cerebral edema and 3 hydrocephalus is convenient since this classification reflects the prognosis as in cases of established cerebral cysticercosis therapy is only palliative Stepien reported on 94 operated patients out of 132 and recorded an improvement or recovery in 75 % of the cases in group 1 53 % in group 2 and only 28 in group 3 Our own case belongs to group 1 in this classification and consequently has a good prognosis

REFERENCES

1 Arseni C & Samitca, D C Cysticercosis of the brain Brit med J 2 494 1957

2 Bojarski Z & Waleszkowski J Cysticercosis of the brain Wiad Parazyt 9 571 1963

3 Dorfsman J The radiologic aspects of cerebral cysticercosis Acta radiol (Stockh) 1 836 1963

4 Heinz, H J & Macnab G M Cysticercosis in the Bantu of Southern Africa S Afr J med Sci 30 19 1965

5 Marsden P D & Hoskins D W Progress in gastroenterology Gastroenterology 51 701 1966

6 McKeown M Cysticercosis J Coll Radiol Aust 8 78, 1964

7 Milenkovic P & Stula D Cysticercosis of the nervous system Report on 17 treated cases Srpski Arkh tselok Lek 9 39 1964

8 Pupo P P Cysticercosis of the nervous system Clinical manifestations Rev Neuro psiquiat 27 70 1964

9 Stepien L Cerebral cysticercosis in Poland J Neurosurg 19 505 1962

10 Stoll N R This wormy world J Parasit 33 1 1947

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A SEVERE HAEMORRHAGIC DISORDER WITH PROLONGED BLEEDING TIME DUE TO A PLASMA DEFECT BUT WITH NORMAL FACTOR VIII

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Abstract A young girl had bleeding symptoms resembling those of severe von Willebrand s disease At 12 years of age she had perilous menorrhagia The Duke bleeding time usually exceeded 30 min but all coagulation factors were normal and unlike what is seen in von Willebrand s disease factor VIII was normal There was no pathologic fibrinolysis The platelet adhesiveness was near the low limit of the normal range when tested with Hellem s whole blood method, and normal when examined with his plasma ADP method, but decreased when assessed with Salzman s method. The platelets appeared normal and aggregated and adhered normally to a glass slide Aggregation after addition of ADP or connective tissue suspension was also normal The prothrombin consumption test was usually normal Fraction I-0 fresh plasma, and stored plasma shortened the bleeding time There was no family history of a bleeding disorder and her parents showed no evidence of such a condition.

The findings suggest a special bleeding disorder of a new type resembling severe von Willebrand s disease but distinctly different from it. We have provisionally called the condition morbus Rita

Congenital haemorrhagic disorders with prolonged bleeding time but normal platelet count may be divided into two main groups one with primary platelet disorders su h as thrombasthenia, and the other with primary plasma defects such as von Willebrand s disease

Von Willebrand s disease is characterized by a dominant heredity a prolonged bleeding time and a low factor VIII (20) Platelet adhesiveness as tested with Hellem s methods is normal (11) but mostly low when tested with Salzman s method (9 31) Platelet function is otherwise normal The prolonged bleeding time can be shortened by administration of fraction I-0 or fresh plasma

Von Willebrand s disease is classified as severe when the Duke bleeding time exceeds 20 minutes otherwise as mild In the severe form factor VIII is as low as 1–20% in the mild form 20–60 (20)

In Sweden von Willebrand s disease has been diagnosed in more than 200 members of 100 families Patients with primary platelet defects of varying severity have also been observed (9 10 12) In addition one patient had a severe bleeding disorder with a markedly prolonged bleeding time but differing in many respects from other known bleeding disorders Factor VIII and other coagulation factors were normal and the defect was corrected by plasma not necessarily fresh Since the patient may have some hitherto unknown type of congenital haemorrhagic disorder the case is reported in detail

MATERIAL AND METHODS

Human fraction I-0 containing AHF (factor VIII) was prepared at Chemistry Department II Karolinska Institutet, Stockholm, Sweden by the glycine method of Blomback and Blomback (4) One dose of fraction I-0 is prepared from 1400 to 1600 ml of fresh normal plasma and contains about 3 g of protein Usually one half of one dose of fraction I 0 was given on each occasion Half a dose of fraction I-0 dissolved in 100 ml of isotonic saline has an AHF activity 5 to 8 times that of 100 ml fresh normal plasma

Collect on of blood was performed in the way described earlier (11)

Coagulation tests All the methods used for preparing the blood samples and for determining the various coagulation factors have been described elsewhere (23) The factor VIII activity of plasma was assessed from its normalizing effect on the recalcification time of haemophilia A plasma (?., 23 4) and the amount of AHF (factor VIII) present was expressed as a percentage of that found in a normal standard consisting of pooled plasma from ten normal individuals

The *ble d ng time* was mostly determined with Duke s method, using standardized haemolets (Dade Reagent, Inc.,

Miami Florida USA) Determinations were performed on both ears Normal range 1 to 4 min Use was also made of the method of Ivy as modified by Borchgrevink and Waaler (5 11 25)

The *fibrinolytic system* was studied in the way described previously (26 27)

Platelet counts were made according to Hellem s modification (16) of Nygaard s method (29) Control counts were also made by the method of Bjorkman (2)

Platelet suspensions were prepared and tested for platelet factors 1 3 and 4 as described previously (28)

Platelet rich plasma was prepared by centrifuging citrated blood (1 part 3 8 sodium citrate dihydrate and 9 parts blood) drawn by the silicon technique at 185 g for 10 min immediately after collection

Platelet deficient plasma was prepared by centrifuging platelet rich citrated plasma at 16 000 g for 30 min in plastic tubes at +4 C and then carefully pipetting off the supernatant plasma without disturbing the layer of platelets

Platelet adhesiveness was measured according to a slight modification (11) of Hellem s whole blood method (16) According to this method citrated whole blood is passed through a column of glass beads and the percentage of adherent platelets is calculated Platelet adhesiveness in *platelet rich plasma after addition of ADP in various con*centrations was determined according to a slight modification (11) of the method of Hellem et al (17) Platelet adhesiveness was also measured with the original method of Salzman (31) according to which whole blood is rapidly passed directly from the vein through a glass filter

Platelet adhesion aggregation and spreading were studied by phase contrast microscopy of a drop of platelet rich citrated plasma placed on a glass slide under a cover glass Both ordinary and siliconized glass slides were used Platelet spreading and aggregation were also investigated by Dr J Scharrer a member of Dr Breddin s staff using Breddin s original methods (6 7)

Platelet aggregation after addition of connective tissue suspension or ADP was studied macroscopically microscopically and with the aid of a photometer (9 12)

Clot retraction was determined in diluted platelet rich plasma according to a modification of the Voss method (12)

The *tourniquet test* was performed by placing a blood pressure cuff around the upper arm and inflating it to a pressure intermediate between the systolic and diastolic pressure The number of punctiform haemorrhages that had appeared within 10 min in an area of 20 cm² was counted. A count of more than 10 was considered positive

CASE REPORT

The patient was a girl born in 1950 Since early childhood she had had an increased bleeding tendency and had bruised readily She had also had gingival bleeding and bled profusely after cuts On several occasions she had had epistaxis requiring cauterization At the age of 10 she was admitted to hospital because of severe bleeding after extraction of a tooth The bleeding time was prolonged despite a normal number of platelets She was then referred to our laboratory for investigation At one determination of the bleeding time the patient bled profusely from the wound for several hours Owing to her severe bleeding tendency severe menstrual bleedings were expected and she was therefore hospitalized at the onset of her first menstruation which occurred when she was 12 years old She then bled profusely for 14 days and the haemoglobin fell from 13 8 g/100 ml to 7 3 g/100 ml She received 3 pints of blood In the following years menorrhagia was very troublesome and on five occasions shock supervened In December 1963 the menstrual flow continued for 14 days She then left hospital but was readmitted some hours later in a state of shock owing to recurrent severe bleeding This recurrence lasted for 3 weeks during which she received all together 5 pints of blood In July 1964 the flow lasted for more than a month and in May June and August 1966 for 2–3 weeks She has been hospitalized on 50 occasions and has received all together 29 pints of blood and 23 half-doses of fraction I 0 She was always on iron therapy but was nevertheless often anaemic (Hb 9–10 g/100 ml) The menstrual flow became less excessive after infusion of plasma or fraction I 0 or treatment with epsilon aminocaproic acid Of this drug the patient has received a total of more than 5000 g and this treatment of the patient has already been reported (19) During the last 12 months she has been successfully treated with gestagens Tooth extraction in 1965 was performed under cover of fraction I 0 and epsilon aminocaproic acid without undue bleeding She never had joint bleeding and was otherwise healthy and well developed

Physical and laboratory investigation

Physical examination revealed no abnormalities Blood pressure 120/70 mm Hg ECG normal Chest X ray showed nothing remarkable ESR 7 mm/h Electrophoresis showed a normal pattern Her haemoglobin level was often low owing to bleeding or iron deficiency but otherwise her blood picture was normal The leucocyte count was about 5000/mm³ The patient belonged to blood group B CDe/cde M+ N+ Ss P(+) K(−) Le(a− b+) Fy(a−) Coombs test was negative No irregular antibodies against red cells or leucocytes could be demonstrated

Widal s Wassermann s Waaler Rose s and Paul Bunnel s tests were negative as were antistreptolysin antistaphylolysin CRP cold agglutination tests and the gonococcal complement binding reaction Listeria agglutination occurred at a dilution of 1 64 and complement binding at 1 5

Coagulation studies

Coagulation analyses on more than 50 occasions in 1960–67 always gave consistent results (Table I) The Duke bleeding time usually exceeded 30 min but was occasionally about 15 min on one side

Table I *Coagulation analysis*

	Patient	Range or standard error	No of investigations	Mother	Father	Maternal grandmother	Normal range
Bleeding time							
Duke min	>30	10–30	30	3–4	–	–	1–4
Ivy min	>30	>30	4	8		15	6–15
Coagulation time							
glass min	13	7–19	9	12	6	1[illegible]	8–14
plastic, min	[illegible]5	17–3[illegible]	7			23	15–[illegible]5
Prothrombin consumption	21	±5 9	18	27		6	0–[illegible]0
One stage prothrombin time sec	16 [illegible]	15 0–17 [illegible]	9		16 8		15–17
F VII + F X + prothrombin (P&P) %	95	75–1[illegible]0	9	80	93		80–1[illegible]0
Recalcification time sec	144	120–180	9		183		1[illegible]0–160
F V	108	80–139	8	85	145		80–1[illegible]0
F VIII	87	±5 4	35	71	1[illegible]0	131	60–160
F IX	8[illegible]	62 105	7	92		95	60–160
F XI	100		[illegible]				60–160
Fibrinogen g/100 ml	0 33	0 [illegible]–0 39	7				0 26–0 34
Plasminogen activity	109	88–131	6				60–140
Urokinase inhibitors	90	77–104	5				60–140
Platelet factors 1 3 4	Normal		[illegible]				
Platelet number per mm³	185 000	±6700	40	238 000		246 000	135 000–300 000
Platelet adhesiveness							
Hellem s whole blood	[illegible]2	±1 2	33				30 S.D. ±4
Plasma + ADP 0 10 µg/ml	47	21–68	3				48 S.D. ±15
0 05 µg ml	3	10–55	3				26 S.D. ±12
Salzman s method	9	3–17	4				0–60

The Ivy bleeding time always exceeded 30 min The coagulation time and the various coagulation factors were normal AHF (factor VIII) determined on 35 occasions was on the average 87% and factors IX XI and XII were normal No increased fibrinolytic activity was demonstrated in the blood samples The patient had never shown signs of circulating anticoagulants

The platelets were always normal in number as well as in appearance in the phase contrast microscope They aggregated and adhered to glass in a normal way or possibly somewhat more strongly this was apparent also when using Bredd[illegible]n s methods Viscous metamorphosis after recalcification was normal Platelet adhesiveness as tested with Hellem s whole blood method was 22 (mean of 33 determinations) i e bordered the lower limit of the normal range After varying doses of ADP platelet adhesiveness according to Hellem s plasma ADP method was normal The value found with Salzman s method was always decreased Platelet aggregation in platelet rich citrated plasma was normal after addition of ADP to a final concentration of 1 µg/ml of ADP The platelets also aggregated normally after addition of connective tissue suspension dilute thrombin trypsin or papain solutions Clot retraction was normal The activity of platelet factors 1 3 and 4 was normal Prothrombin consumption was mostly normal

Familial investigation

Neither of the parents had had any bleeding symptoms and laboratory studies revealed nothing remarkable The patient s maternal grandmother was also investigated and found to be healthy There was no relative with a history of haemorrhagic diathesis The patient had no sisters or brothers

Therapeutical trials

The patient was repeatedly treated with whole blood and various kinds of plasma and plasma derivatives (Table II) Fraction I-0 shortened the Duke bleeding time and decreased clinical bleeding The effect of one dose of fraction I-0 did not exceed 24 h. After the infusion the increase of factor VIII was that expected from the amount of factor VIII administered and there was no retarded increase The administration of 400–1000 ml of fresh or stored plasma had a similar effect

Table II *Effect of various infusions on the bleeding time of the patient*

Blood derivate	Dose (ml)	Total number of infusions	Effect		
			Good[a]	Moderate[b]	Bad[c]
Fraction I-O	100-200	11	8	3	0
Fresh B plasma	400-500	2	2	0	0
Stored B plasma	400-1000	9	8	0	1
Stored blood	450-900	5	2	1	2
Red cell concentrate	450-900	3	0	1	2
B serum	400-500	3	0	0	3
Platelet suspension	4 donors	1	0	0	1
Fresh plasma mixed blood group	300-500	4	0	1	3
Stored plasma mixed blood group	400-1000	11	4	6	1
Fresh B plasma from von Willebrand patient	400	1	0	0	1
Infusion of further normal stored B plasma	200	1	0	1	0
Stored O plasma of low antibody titre	550	1	0	1	0
Fresh frozen B plasma from patient with thrombasthenia	700	1	0	1	0
Stored A plasma adsorbed with red cells of blood group B	900	1	0	0	1
Albumin	5 g	2	0	0	2

[a] Bleeding time shorter than 15 min in both ears
[b] Bleeding time 15–30 min
[c] Bleeding time exceeding 30 min for both ears.

which however was better and more consistent when the plasma belonged to the same blood group as the patient. Fresh plasma did not have a stronger effect than stored plasma Occasionally a urticarial reaction occurred after the plasma infusion and then the bleeding time was never shortened Serum red cell concentrates or a platelet suspension from 4 donors were without Fresh plasma 400 ml from two patients with von Willebrand s disease and belonging to the same blood group was infused without subsequent shortening of the bleeding time As the dose might have been too small a further 200 ml of normal stored plasma from healthy donors belonging to the same blood group were later infused after which the bleeding time for one ear fell to 17 min

Epsilon aminocaproic acid in a dose of 6 g four times a day had a favourable effect on her menstrual bleedings but not on the bleeding time Treatment with prednisone 5 mg three times a day for one month did not affect her bleeding time or bleeding tendency During the last 12 months she has been treated with gestagens Anovlar mite (norethisteron 3 mg ethinyloestradiol 0 05 mg) and the menstrual flow is no longer so profuse but the prolonged bleeding time and low adhesiveness with Salzman s method are unchanged

DISCUSSION

The patient described had a severe haemorrhagic diathesis characterized by menorrhagia nose bleeding and severe bleeding after cuts and dental extractions The Duke and Ivy bleeding times were markedly prolonged but the coagulation time prothrombin consumption and all coagulation factors were normal There was no increased fibrinolysis Platelet adhesiveness was low according to Salzman s method and near the lower limit of the normal range when tested with Hellem s method but the number appearance and other reactions of the platelets were normal or if anything increased When a drop of platelet rich citrated plasma was placed on a glass slide the platelets showed normal or increased adhesion and spontaneous aggregation Aggregation with ADP and connective tissue suspension was also normal Platelet factors were normal Viscous metamorphosis and clot retraction were also normal Although the disease was obviously congenital examination of her parents revealed nothing remarkable and no relative was known to have exhibited any bleeding symptoms The Duke bleeding time and the bleeding symptoms decreased on treatment with fraction I-0 fresh or stored plasma especially when the plasma originated from a donor of the same blood group

The fact that platelet aggregation and clot retraction were normal excluded Glanzmann's severe thrombasthenia The normal prothrombin consumption and normal factor 3 and the good response to plasma infusions argue against thrombopathy or other primary platelet disorders A prolonged bleeding time in association with decreased factor IX or XI has been described (3 8 13 14 15 30 32 33 34) but these factors were normal in this patient Recently Hirsh et al (18) described a patient with prolonged bleeding time and an abnormal response to connective tissue suspension but the possibility of such conditions was ruled out in our patient

The condition resembled the severe form of von Willebrand's disease but not in all respects There was thus no dominant heredity factor VIII was normal and in contrast with what might be expected from our experiences with patients with von Willebrand's disease the condition responded also to stored plasma In mild von Willebrand's disease a normal factor VIII is occasionally found but the prolongation of the bleeding time the bleeding tendency and the decrease in factor VIII are proportional and in a patient like ours a very low level of factor VIII was expected No retarded increase in factor VIII was observed in the infusion trials

It may therefore be concluded that the patient had a special hitherto unrecognized congenital bleeding disorder resembling but not identical with von Willebrand's disease We have provisionally called the condition Morbus Rita after the Christian name of the patient. The symptoms laboratory findings and presence of the factor in fraction I-0 suggest that the disorder might be an isolated lack of the von Willebrand factor and the results of a trial in which plasma from von Willebrand patients failed to shorten the bleeding time argue for this possibility However the results of a single trial should not be overestimated The earlier finding that infusion of plasma from haemophilic patients raised the level of factor VIII in patients with von Willebrand's disease (21) suggests that the von Willebrand factor occupies an earlier position in a sequence of reactions than factor VIII and is of importance for the normal level of this factor As factor VIII was normal in this patient the von Willebrand factor should therefore be normal too but some factor specifically responsible for maintenance of a normal bleeding time and activated by the von Willebrand factor might be missing Other mechanisms are also possible but investigations to elucidate the question were hampered by lack of appropriate in vitro methods to test the von Willebrand factor No infusion of plasma from the patient was given to patients with von Willebrand's disease

An interesting observation was that plasma from a donor belonging to the same blood group was more effective than mixed plasma This suggests that the factor might possibly be inactivated in immunological reactions The possibility of a connection between complement and opsonizing factors and factors associated with platelet adhesion and aggregation in the early stages of haemostasis has been suggested by Bettex-Galland and Luscher (1) but the experimental and clinical evidence available in support of such an idea is so far meagre

Bleeding should be treated by infusion of whole blood fraction I-0 or plasma from a donor of the same blood group as the patient Epsilon aminocaproic acid depresses the local fibrinolysis normally present in many tissues and controls menorrhagia The menstrual bleeding responds also to gestagens

ACKNOWLEDGEMENTS

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REFERENCES

1 Bettex-Galland M & Luscher E F Untersuchungen uber die Auslosung der viskosen Metamorphose der menschlichen Blutplattchen durch Immunkomplexe Path Microbiol (Basel) 7 533 1964
2 Bjorkman S E A new method for enumeration of platelets Acta haemat 22 377 1959
3 Blackburn, E K Monaghan J H Lederer H & MacFie J M Christmas disease associated with primary capillary abnormalities Brit med J 1 154 196
4 Blomback B & Blomback M Purification of human and bovine fibrinogen Arkiv Kemi 10 415 1956
5 Borchgrevink C F & Waaler B A The secondary bleeding time A new method for the differentiation of haemorrhagic diseases Acta med scand 16 36.. 1958
6 Breddin K & Bauke J Thrombozytenagglutination und Gefasskrankheiten Blut 11 144 1965
7 Breddin K & Burck K H Zur Klinik der Thrombozytenfunktionsstorungen unter besonderer Berucksichtigung der Ausbreitungsfahigkeit der Thrombozy

ten an silikonisierten Glasflachen Thrombos Diathes haemorrh (Stuttg) 9 575 1963
8 Combrisson A Le Bolloch A G Debray J & Benhamou J P Deficit en facteur antihemophilique B associe à un allongement du temps de saignement Sang 28 137 1957
9 Cronberg S To be published
10 Cronberg S & Nilsson I M Investigations in a family with thrombasthenia of moderately severe type with 16 affected members Scand J Haemat. 5 17 1968
11 *Cronberg S Nilsson I M & Silwer J Studies* on the platelet adhesiveness in von Willebrand s disease Acta med scand 180 43 1966
12 Cronberg S Nilsson I M & Zetterqvist E Investigation of a family with members with both severe and mild degree of thrombasthenia Acta paediat scand 56 189 1967
13 Dormandy K Hardisty R M & MacPherson J Christmas disease and capillary abnormality Brit med J 1 566 1962
14 Gobbi F Antonucci M & Ascari E Angioemofilia B Haemat lat 3 363 1960
15 Gugler E Angiohamophilie Schweiz med Wschr 90 534 1960
16 Hellem A J The adhesiveness of human blood platelets in vitro Scand J clin Lab Invest Suppl 51 1960
17 Hellem A J Ödegaard A E & Skålhegg B A Investigations on adenosine diphosphate (ADP) induced platelet adhesiveness in vitro Part 1 The ADP platelet reaction in various experimental conditions Thrombos Diathes haemorrh (Stuttg) 10 61 1963
18 Hirsh J Castelan D J & Loder P B Spontaneous bruising associated with a defect in the interaction of platelets with connective tissue Lancet 2 18 1967
19 Nilsson I M & Bjorkman S E Experiments with epsilon aminocaproic acid (EACA) in the treatment of profuse menstruation Acta med scand 177 445 1965
20 Nilsson I M & Blomback M Von Willebrand s disease in Sweden—occurrence pathogenesis and treatment Thrombos Diathes haemorrh Suppl 2 103 1962
21 Nilsson, I M Blomback M & Blomback B v Willebrand s disease in Sweden Its pathogenesis and treatment Acta med scand 164 263 1959
22 Nilsson I M Blomback, M & Francken I von On an inherited autosomal haemorrhagic diathesis with antihemophilic globulin (AHG) deficiency and prolonged bleeding time Acta med scand 159 35 1957
23 Nilsson I M Blomback M & Ramgren O Haemophilia in Sweden I Coagulation studies Acta med scand 170 665 1961
24 Nilsson I M Blomback M Ramgren O & Francken I von Haemophilia in Sweden II Carriers of haemophilia A and B Acta med scand 171 223 1962
25 Nilsson I M Magnusson S & Borchgrevink C The Duke and Ivy methods for determination of the bleeding time Thrombos Diathes haemorrh (Stuttg) 10 233 1963
26 Nilsson I M & Olow B Determination of fibrinogen and fibrinogenolytic activity Thrombos Diathes haemorrh (Stuttg) 8 297 1962
27 — Fibrinolysis induced by streptokinase in man Acta chir scand 123 247 1962
28 Nilsson I M Skanse B Bjorkman S E & Serin F Platelet function in thrombocythaemia The effect of platelets and serotonin on serum potassium and bilirubin Acta med scand 167 353 1960
29 Nygaard K K A direct method of counting platelets in oxalated plasma Proc Mayo Clin 8 365 1933
30 Ottaviani P Mandelli F & Deriu L La proprieta adesiva delle piastrine nell angiohemofilia B Progr med (Naples) 19 370 1963
31 Salzman E W Measurement of platelet adhesiveness A simple in vitro technique demonstrating an abnormality in von Willebrand s disease J Lab clin Med 62 724 1963
32 Sjølin K E & Videbæk A Christmas factor deficiency and decreased capillary resistance in a female with haemorrhagic diathesis Dan med Bull 3 *85 1956*
33 Soulier J P & Larrieu M J Deficit en facteur antihemophilic B avec allongement du temps de saignement Sang 28 138 1957
34 White J G Yunis E Collhander M & Krivit, W Prolonged bleeding time in a patient with plasma thromboplastin antecedent deficiency Observations on correction of the bleeding time by platelet transfusion J Paediat 63 1081 1963

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TURNOVER OF ^{131}I AND ^{125}I LABELLED HAPTOGLOBIN IN MAN

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Abstract Haptoglobin catabolism has been studied in 17 subjects with the aid of ^{131}I and ^{125}I labelled haptoglobin The catabolism has been found to be in the order of 10–20% of the i.v. pool per day—a catabolic rate significantly lower than that reported by previous investigators

Plasma haptoglobin concentration varies widely in disease It may be zero in hemolytic disease and is increased sometimes considerably in conditions with inflammation or tissue breakdown such as infarctions collagen disease and malignant neoplasms (11 13) Haptoglobin metabolism has been studied mainly in connection with increased red cell destruction a condition in which plasma haptoglobin exerts its physiological effect of binding plasma hemoglobin thus preventing it from being excreted by the kidneys (2 3 9 12) The possible importance of the *increased* haptoglobin levels in the other conditions mentioned is however entirely unknown

We have studied the turnover rate of iodinated haptoglobin in hospitalized patients without signs of increased hemolysis

MATERIAL AND METHODS

All subjects were hospitalized for at least three weeks during the study Those labelled "normals" were patients admitted for routine investigations None hade signs of anemia or increased erythrocyte breakdown nor of recent acute disease The ESR was normal or only slightly elevated (Table I) Patients all with elevated ESR, had the following diagnoses rheumatoid arthritis two recent myocardial infarction one gastric ulcer one acute gall bladder disease one carcinoma of the colon one acute deep vein thrombosis one None had signs of increased hemolysis hemoglobins—with one exception—were within normal limits

To ensure complete urine collection indwelling catheters were used All subjects were given Lugol's solution (10 drops 3 times daily) at least three days before the start of the study The radioiodinated haptoglobin preparation was injected intravenously Blood samples were drawn 10 min after the injection and later every day at approximately the same time of the day Urine was collected in 24 h samples

Haptoglobin was fractionated from normal human plasma by the method of Steinbuch and Quentin (14) Further separation to obtain single polymers was done on Sephadex G 200 (3) Iodination was performed essentially according to the iodomonochloride technique of McFarlane (7) After iodination sterile filtration was performed through glass filters and tests were made to ensure that the preparations were sterile and pyrogen free The hemoglobin binding capacity was tested after these procedures and was found to be unaltered As a measure of catabolism the U P ratio was used i.e. the fraction of the dose excreted in the urine (= U) during 24 hours divided by the mean value of the fraction of the dose in the plasma pool (= P) during the same time according to principles set down by Campbell et al (1)

Haptoglobin concentration was determined by Jayle's method as described by Nyman (11)

Alpha$_2$-globulin concentration was determined from paper electrophoresis

RESULTS

The first set of experiments (A) was performed with ^{131}I the second and the third (B and C) with ^{125}I The slowly falling value of the catabolic rate (Fig 1) in the first two sets (A and B) seems to show that at least some denaturation had occurred The last two experiments (C) however have given absolutely constant catabolic rates indicating that the haptoglobin preparation was undenaturated Table I summarizes the results Fig 2 shows the typical disappearance curve in one of the last two experiments

The haptoglobin catabolism in the first series of experiments amounted to on an average 20 of the i.v. pool per 24 hours In the last two

Table I *Clinical data and results of haptoglobin catabolism studies in ten normal subjects and seven patients*

Pat. no	Sex	Age	Diagnosis	Hb (g/100 ml)	ESR (mm/h)	Serum haptoglobin (mg/100 ml)	α_2 globulin (g/100 ml)	Catabolism i v pool/day (%)	Catabolism (g/day)
(A) ^{131}I									
1	o	48	Normal	13 8	12	—	—	16 3	
2	o	52	Normal	14 7	5	—	—	11 0	
3	♂	57	Normal	13 8	11	—	—	12 8	
4	♀	36	Normal	10 3	8	—	—	17 3	
5	♀	74	St.p myocardial infarction	12.7	42	—	—	18 7	
6	♂	4.	Gastric ulcer	11 7	56	—	—	24 9	
7	♀	49	Acute arthritis (rheumatoid?)	10 7	96	—	—	23 6	
							Mean	17 8	
							Range	11 0–24 9	
(B) ^{125}I									
8	♀	83	Normal	13 2	38	160	0 45	18 4	0 7
9	o	66	Normal	14 0	3	79	0 45	21 4	0 5
10	o	56	Normal	14 3	27	244	0 56	20 8	2.1
11	♀	46	Normal	15 8	6	178	0 56	21 1	1 1
12	♀	64	Rheumatoid arthritis	11 9	102	363	0 89	17 2	2 4
13	♂	59	Leg thrombosis	11 4	98	342	—	25 2	3 5
14	♀	57	Colon carcinoma	8 8	72	300	—	21 3	1 6
15	♂	53	Acute gall bladder disease	11 4	110	600	1 05	21 9	5 1
							Mean	20 9	
							Range	17 2–25 2	
(C) ^{125}I									
16	♂	80	Normal (st.p slight cerebral vascular accident)	13 2	19	104	0 57	8 5	0 4
17	♀	81	Normal" (st.p slight cerebral vascular accident)	12 3	30	124	0 70	13 5	0 4

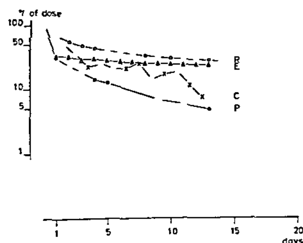


Fig 1 Haptoglobin catabolism (case 5) *R* retained dose *E* extravascular pool *P* plasma disappearance curve *C* catabolic rate (U/P) per cent per 24 hours (cf Methods)

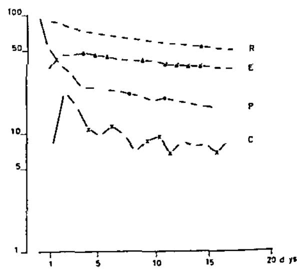


Fig 2 Haptoglobin metabolism in a normal man (case 16) Constant catabolic rate

experiments the rate of catabolism was found to be lower 9 and 14 % respectively

DISCUSSION

As stated in the introduction haptoglobin hemo globin complex metabolism has been studied by several workers (2 4 6) However Freeman (3) found that haptoglobin catabolism was much less only one third of hemoglobin catabolism and concluded that it was possible that normally all haptoglobin was catabolised by a non specific mechanism without combination with hemoglobin Garby and Noyes (5) in 1966 stated that the hemoglobin haptoglobin complex pathway was responsible for about half the total haptoglobin catabolism and in 1967 after further studies that 50–80 % of the total catabolism of hapto globin occurs by way of the hemoglobin path way (10)

The purpose of this study was to analyze the haptoglobin catabolism in normals and in patients with elevated serum haptoglobin levels—without signs of increased red cell destruction As em phasized by Freeman (3) the plasma concentra tion of any protein is a balance between synthesis and catabolism The plasma concentration rises markedly in inflammatory conditions among others It is unknown whether this increase has any physiological meaning

Nyman (11) measured the disappearance of plasma haptoglobin after acute pneumonia in nine patients She calculated a half life" for excess haptoglobin of 5 4 days (range 4 4–6 8) Moretti et al (8) reported a haptoglobin half life of 3 5 and 4 days in two subjects given ^{131}I labelled haptoglobin Freeman (3) in his study with ^{1} I labelled haptoglobin found an average catabolism of 36 % of the i v pool per day (range 19–55) in normals and an increase up to 500 % i v catabolism in patients with acute hemolysis In non hemolytic conditions he found no correlation between haptoglobin concentration and either hemoglobin concentration or rate of haptoglobin catabolism

In the majority of our experiments the average catabolism amounted to 20 % of the i v pool per day (corresponding to a half life of 3 7 days) As stated above the slowly decreasing value of the catabolic rate (cf Fig 1) in experiments A and B might indicate that some denaturation had taken place This type of curve however does not necessarily indicate denaturation but could be a sign of individual variations in the handling of foreign" proteins In no experiments were there found any signs of strong denaturation with massive excretion during the first two days of the experiment

In the last two experiments (C) the catabolic rate was absolutely constant It was found to be lower than in the previous experiments amounting to only 8 5 and 13 5 % of the i v pool per day

All the results would indicate a slower catab olism of haptoglobin than that found by Free man (3) Against his average catabolism of 36 % we found a catabolic rate between 10 and 20 % Even admitting some denaturation in some of the experiments this would be a significant differ ence As Freeman (3) points out alteration of a protein during laboratory procedures will always induce an elevated never a decreased catabolism The low figures found here would therefore seem to be reliable

The differing results cannot be ascribed to dif ferences in the patient material as the composi tion seems to be very similar in the two groups studied by Freeman (3) and ourselves It should be added that all subjects labelled normals were in steady state during the study

The lowest catabolic rate was found in two elderly subjects No studies however have been published to indicate that protein metabolism would decrease with age

In the patients with elevated ESR the hapto globin catabolism was slightly more rapid (mean 22 range 17–25 per cent) than in the normal subjects (mean 16 range 9–21 per cent) This difference however was not significant

REFERENCES

1 Campell R M Cuthbertson D P Matthews C M & McFarlane A S Int J appl Radiat 1 66 1956
2 Faulsteck D A Lowenstein J & Yiengst, M J Blood 0 65 196
3 Freeman T In Protides in biolog cal fluids (ed H Peeters) p 344 Elsevier Amsterdam 1964
4 Garby L & Noyes W J clin Invest 38 1479 1959
5 — In Protides in biolog cal fluids (ed H Peeters) p 41 Elsevier Amsterdam 1966
6 Laurell C B & Nyman M Blood 16 493 1957
7 McFarlane A S Nature (London) 18 53 1958

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2	♂	52	Normal	14 7	5	—
3	♂	57	Normal	13 8	11	—
4	♀	36	Normal	10 3	8	—
5	♀	74	St p myocardial infarction	12 7	42	—
6	♂	42	Gastric ulcer	11 7	56	—
7	♀	49	Acute arthritis (rheumatoid?)	10 7	96	—
(B) ^{125}I						
8	♀	83	Normal	13 2	38	160
9	♂	66	Normal	14 0	3	79
10	♂	56	Normal	14 3	27	244
11	♀	46	Normal	15 8	6	178
12	♀	64	Rheumatoid arthritis	11 9	102	363
13	♂	59	Leg thrombosis	11 4	98	342
14	♀	57	Colon carcinoma	8 8	72	300
15	♂	53	Acute gall bladder disease	11 4	110	600
(C) ^{125}I						
16	♂	80	Normal (st p slight cerebral vascular accident)	13 2	19	104
17	♀	81	Normal (st p slight cerebral vascular accident)	12 3	30	124

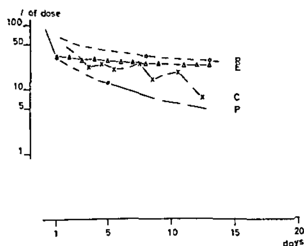


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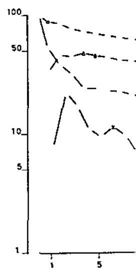


Fig 2 Haptoglobin m 16) Constant catabolic r

experiments the rate of catabolism was found to be lower 9 and 14 respectively

DISCUSSION

As stated in the introduction haptoglobin hemoglobin complex metabolism has been studied by several workers (2 4 6) However Freeman (3) found that haptoglobin catabolism was much less only one third of hemoglobin catabolism and concluded that it was possible that normally all haptoglobin was catabolised by a non specific mechanism without combination with hemoglobin Garby and Noyes (5) in 1966 stated that the hemoglobin haptoglobin complex pathway was responsible for about half the total haptoglobin catabolism and in 1967 after further studies that 50–80% of the total catabolism of haptoglobin occurs by way of the hemoglobin pathway (10)

The purpose of this study was to analyze the haptoglobin catabolism in normals and in patients with elevated serum haptoglobin levels—without signs of increased red cell destruction As emphasized by Freeman (3) the plasma concentration of any protein is a balance between synthesis and catabolism The plasma concentration rises markedly in inflammatory conditions among others It is unknown whether this increase has any physiological meaning

Nyman (11) measured the disappearance of plasma haptoglobin after acute pneumonia in nine patients She calculated a half life for excess haptoglobin of 5 4 days (range 4 4–6 8) Moretti et al (8) reported a haptoglobin half life of 3 5 and 4 days in two subjects given ^{131}I labelled haptoglobin Freeman (3) in his study with ^{1} I labelled haptoglobin found an average catabolism of 36 of the i v pool per day (range 19–55) in "normals and an increase up to 500 / i v catabolism in patients with acute hemolysis In non hemolytic conditions he found no correlation between haptoglobin concentration and either hemoglobin concentration or rate of haptoglobin catabolism.

In the majority of our experiments the average catabolism amounted to 20 of the i v pool per day (corresponding to a "half-life" of 3 7 days) As stated above the slowly decreasing value of the catabolic rate (cf Fig. 1) in experiments A and B might indicate that some d naturation had taken place This type of curve however does not necessarily indicate denaturation but could be a sign of individual variations in the handling of foreign proteins In no experiments were there found any signs of strong denaturation with massive excretion during the first two days of the experiment

In the last two experiments (C) the catabolic rate was absolutely constant It was found to be lower than in the previous experiments amounting to only 8 5 and 13 5% of the i v pool per day

All the results would indicate a slower catabolism of haptoglobin than that found by Freeman (3) Against his average catabolism of 36% we found a catabolic rate between 10 and 20 Even admitting some denaturation in some of the experiments this would be a significant difference As Freeman (3) points out alteration of a protein during laboratory procedures will always induce an elevated never a decreased catabolism The low figures found here would therefore seem to be reliable

The differing results cannot be ascribed to differences in the patient material as the composition seems to be very similar in the two groups studied by Freeman (3) and ourselves It should be added that all subjects labelled normals were in steady state during the study

The lowest catabolic rate was found in two elderly subjects No studies however have been published to indicate that protein metabolism would d crease with age

In the patients with elevated ESR the haptoglobin catabolism was slightly more rapid (mean 22 range 17–25 per cent) than in the normal subjects (mean 16 range 9–21 per cent) This difference however was not significant

REFERENCES

1 Campell R M Cuthbertson D P Matthews C M & McFarlane A S Int J appl Radiat 1 66 19 6
2 Faulsteck D A Lowenste n J & Yiengst, M J B ood 20 65 1962.
3 Freeman T In Prot des in biological flu ds (ed H Peeters) p 344 Elsevier Amsterdam 1964
4 Garby L & Noyes W J clin In est. 38 1479 1959
5 In Prot i les in biolog al fluids (ed H Peeters) p 41 Elsevier Amsterdam 1966
6 Laurell C B & Nyman, M Blood 16 493 1957
7 McFarl n A S Nature (London) 18 53 1958

Table I *Clinical data and results of haptoglobin catabolism studies in ten normal subjects and seven patients*

Pat no	Sex	Age	Diagnosis	Hb (g/100 ml)	ESR (mm/h)	Serum haptoglobin (mg/100 ml)	α_2 globulin (g/100 ml)	Catabolism i v pool/day ( )	Catabolism (g day)
(A) ^{131}I									
1	♂	48	Normal	13 8	12	—	—	16 3	
2	♂	52	Normal	14 7	5	—	—	11 0	
3	♂	57	Normal	13 8	11	—	—	12 8	
4	♀	36	Normal	10 3	8	—	—	17 3	
5	♀	74	St p myocardial infarction	12 7	42	—	—	18 7	
6	♂	42	Gastric ulcer	11 7	56	—	—	24 9	
7	♀	49	Acute arthritis (rheumatoid?)	10 7	96	—	—	23 6	
							Mean	17 8	
							Range	11 0–24 9	
(B) ^{125}I									
8	♀	83	Normal	13 2	38	160	0 45	18 4	0 7
9	♂	66	Normal	14 0	3	79	0 45	21 4	0 5
10	♂	56	Normal	14 3	27	244	0 56	20 8	2 1
11	♀	46	Normal	15 8	6	178	0 56	21 1	1 1
12	♀	64	Rheumatoid arthritis	11 9	102	363	0 89	17 2	2 4
13	♂	59	Leg thrombosis	11 4	98	342	—	25 2	3 5
14	♀	57	Colon carcinoma	8 8	72	300	—	21 3	1 6
15	♂	53	Acute gall bladder disease	11 4	110	600	1 05	21 9	5 1
							Mean	20 9	
							Range	17 2–25 2	
(C) ^{125}I									
16	♂	80	Normal (st p slight cerebral vascular accident)	13 2	19	104	0 57	8 5	0 4
17	♀	81	Normal (st p slight cerebral vascular accident)	12 3	30	124	0 70	13 5	0 4

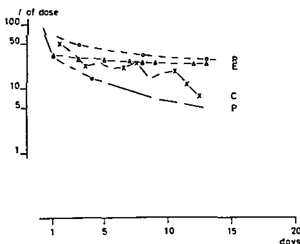


Fig 1 Haptoglobin catabolism (case 5) *R* retained dose *E* extravascular pool *P* plasma disappearance curve *C* catabolic rate (U/P) per cent per 24 hours (cf Methods)

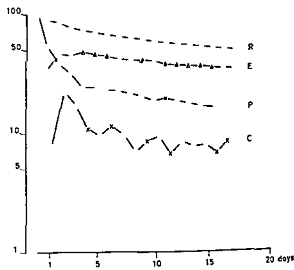


Fig 2 Haptoglobin metabolism in a normal man (case 16) Constant catabolic rate

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STUDIES OF THE CLINICAL AND METABOLIC EFFECTS OF PHLEBOTOMY TREATMENT IN PORPHYRIA CUTANEA TARDA

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Abstract The size of the iron stores and the effect of phlebotomy treatment has been evaluated in twelve patients with porphyria cutanea tarda Clinical and biochemical remission was obtained in all patients The mean treatment period was 4 /4 months in patients who were phlebotomized frequently and were not given iron In two patients who received iron supplements in conjunction with phlebotomy treatment remission was not obtained until the iron administration was stopped In two other patients iron administration after induced remission was associated with relapses No relapse was seen in the others The effect of the treatment is probably due mainly to the reduction of the iron stores Beside the disappearance of active skin symptoms and strongly diminished porphyrin excretion the treatment was associated with improved liver function tests

Porphyria cutanea tarda symptomatica (PCT) is clinically characterized by skin fragility and photosensitivity Blisters often appear on sun exposed areas These frequently ulcerate and lead to scar formation Hypertrichosis and hyperpigmentation are common

Chemically the disease is characterized by the excretion of large amounts of uroporphyrin (UP) and lesser amounts of coproporphyrin (CP) in the urine The excretion of delta aminolevulinic acid (ALA) may be slightly increased but the excretion of porphobilinogen (PBG) is normal The faecal excretion of CP and protoporphyrin (PP) is usually slightly increased

Laboratory signs of disturbed liver function (35) are usually present and decreased glucose tolerance (32) is common Microscopic examination of liver biopsy specimens often reveals steatosis fibrosis or cirrhosis (35)

The disease generally manifests itself in middle or late life its peak incidence being in the fifth decade Males are much more commonly affected than females PCT is usually considered to be acquired and according to the literature most patients are alcoholics In some cases however there is a family history (9 10) Watson (37) has stressed the importance of constitutional (hereditary?) factors that appear to be necessary for the manifestation of the disease

Earlier treatment of the disease has been entirely symptomatic The importance of forbidding alcohol consumption is usually stressed Treatment with chelating agents (24) chloroquine (7) cholestyramine (31) and adenosine monophosphate (8) has been reported to be associated with improvement of cutaneous symptoms in some cases

Ippen was the first to report beneficial effects of phlebotomy treatment in PCT (14 16) Similar results have been reported by others (6 17 29 30) In most series however it is difficult to evaluate the effectiveness of phlebotomy treatment as other factors including stopping drinking and hospitalization may have resulted in improvement of the disease Furthermore the way in which phlebotomy treatment affects the disease and particularly whether the effect of the treatment is due to reduction of iron stores in the liver or to an increase in hemoglobin production is not known Increase of histochemically demonstrable iron in liver cells has been reported (3 34) The estimation of histochemically demonstrable iron in the liver is however an unreliable measure of total liver iron concentration (39)

The main aim of the present study was to evaluate the effectiveness of phlebotomy treatment to quantitate the iron stores in PCT and to determine whether the effect of phlebotomy treatment is related to reduction of the iron stores Furthermore to study the biochemical changes

Table I *Duration of clinically manifest disease at the start of treatment alcohol consumption and porphyrin excretion before and after treatment*

				Alcohol consumption (l abs alcohol/month)		Porphyrin excretion before and after treatment							
						Urine (µg/24 h)				Faeces (µg/g dry wt)			
						UP (N v <100)		CP (N v <150)		CP (N v <20)		PP (N v <40)	
Pat	Sex	Age (y)	Duration (y)	Before treatment	During treatment	Before	After	Before	After	Before	After	Before	After
E S	♂	47	4½	0 9	0 2	4 280	340	160	20	158	8	127	25
H P	♂	42	2¾	1 4	0 3	2 800	70	360		28	2	7	3
S B	♂	53	½	0 8	0 8	2 800	370	110	150	82	5	85	21
G S	♂	50	14	1 6	1 6	3 180	240	510	50	128	22	129	25
G Å	♂	50	2	4 5	2 5	3 420	310		40				
T S	♂	49	1	0 2	0 0	2 080	220	230	40	115		92	
B C	♂	64	1	0 6	0 6	10 970	530	530	20	134		186	
J K	♂	50	1½	0 0	0 0	6 330	800	340	50				
H G	♂	57	¾	0 6	0 6	2 420	730	250	50	8		15	
A G	♀	40	2½	0 2	0 0	3 510	400	460	160	80	13	73	7
J E	♀	64	5	0 0	0 0	4 720	940	380	40	352	2	311	6
M I	♀	23	½	1 5	0 2	3 620	710	130	30	78		83	
Mean		49	3			4 180	470	315	59	116		111	
Range						10 970–2 080	940–70	530–110	160–20	352–8		311–7	

occurring during phlebotomy treatment with respect to porphyrin excretion liver function and glucose tolerance

Twelve patients with typical PCT of whom six did not alter their alcohol consumption were studied The hospitalization was short and no other form of treatment for their disease was given

MATERIAL

The series includes nine men and three women (Table I) all with typical clinical and biochemical signs of PCT Their ages ranged from 23 to 64 years (mean 49 years) Skin fragility and ulcers were consistent symptoms Most patients had vesicles Gross sclerodermal changes were seen only in one patient (J E) Two patients (B C J K) had overt diabetes mellitus requiring treatment.

The patients were questioned about alcohol consumption several times Except for one (B C) reliable information probably has been obtained The amount of alcohol consumed (Table I) has been transformed to absolute alcohol One patient (J E) was a teetotaler and one patient (J K) consumed insignificant amounts Moderate quantities were consumed by two patients (A G T S) The rest of the patients consumed large quantities but none was alcoholic from the social point of view Only one consumed significant quantities of wine (H P)

The youngest of the patients (M I) was started on norethindrone (2 mg daily) and mestranol (0 1 mg daily) as contraceptive agents in November 1965 Skin symptoms appeared in July 1966 The hormone treatment was stopped in September 1966 and the phlebotomy treatment was started five months later It is possible that the treatment with anticonceptive agents may have elicited the disease Appearance of porphyric disease during sex hormone treatment has been reported (41)

The patient (J E) who never had consumed alcohol had epileptic attacks and had been treated with hydantoin derivatives for several years One patient (B C) was being treated with chlorpropamide and one (J K) with tolbutamide One patient (H P) probably had infectious hepatitis 15 years ago No patient had been treated with antiluetic agents

Two patients (G S G Å) were identical twins and have been reported on earlier by Hæger Aronsen (9) Otherwise there was no family history suggestive of porphyria

METHODS

Urinary excretion of UP and CP was determined by the technique of Askevold (1) To ensure conversion of CP precursor to CP iodine wash according to Watson et al (36) was used The urine was collected in bottles supplied with 5 g of sodium carbonate and stored cold or frozen Quantitative determinations of CP and PP in faeces were made according to the method of Holti et al (11) The calculations were made by the formula given by Rimington and Sveinsson (26) The extinction constants and corrections recommended by With (40) were used Quantitative urinary ALA and PBG determinations were performed according to Mauzerall and Granick (23)

Bilirubin and alkaline phosphatases in serum were determined according to a modification by Roos (27)

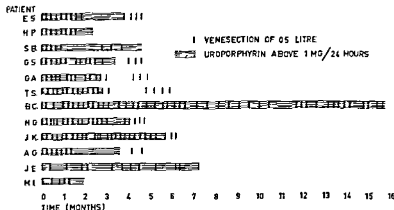


Fig. 1. Phlebotomy program and time required until the UP excretion in urine had fallen to one mg per day. B. C. was given iron sulphate orally (300 mg day) for 6 ½ months. H. G. was given iron dextran intravenously during a period of 10 weeks and the amount given was equal to the calculated iron content of the removed blood (2700 mg).

thymol turbidity according to MacLagan ( ). Serum glutamic oxaloacetic transaminase (SGOT) was determined in a Technicon autoanalyzer according to a modification of the method of Babson et al. (2). The bromsulphalein dye retention test (BSPR) was carried out by the technique of Rosentahl and White (8) but 5 mg of the dye per kg body weight was given and the retention was determined after 45 min. Intravenous glucose tolerance test was performed according to Ikkos and Luft (13).

Hemoglobin was determined photometrically as cyanmethemoglobin. Serum iron (Fe/s) was determined by the method of Laurell (38). The total iron binding capacity of serum (TIBC) was determined according to Peters et al. (5). The desferrioxamine test was performed according to Lundvall and Weinfeld (0). Ten mg of desferrioxamine per kg body weight was administered intramuscularly and the desferrioxamine induced iron excretion (Fe/urine) was determined in urine collected for 24 hours after the desferrioxamine injection.

Liver biopsy was performed in ten patients. In two it had been performed some years before this study (G. S. 1960, H. P. 196). In the last patient (H. P.) the biopsy specimen was taken surgically at operation for duodenal ulcer (subtotal gastrectomy). Otherwise the biopsy specimens were obtained by the percutaneous aspiration technique. A small piece of the specimen was fixed in ten per cent neutral formaldehyde, embedded in paraffin and made into 3 μ sections for histological examination. Hemosiderin was stained with potassium ferrocyanide according to Hutchison (12) and graded on an arbitrary scale of 0–4+ according to the principles previously described for bone marrow sections (19). The major part of the biopsy was freeze-dried and after determination of dry weight analysed for its total non hemin iron content essentially according to the method previously described (38). To render analysis of the small material obtained by percutanous liver biopsy (mean dry weight about 10 mg) possible the extracts were diluted to 10 ml. Proportionally less of the reagents were used.

The phlebotomy treatment (Fig. 1)

Blood was removed by serial phlebotomies of 500 ml each. At each phlebotomy hemoglobin was determined. Serum iron and TIBC were determined at each or every other phlebotomy. The phlebotomies were repeated at weekly intervals until the hemoglobin concentration one week after the last phlebotomy had fallen to 11.0 g per 100 ml or below this level. When this range was reached the hemoglobin concentration and the serum iron were determined weekly for three or four weeks. If during this period the hemoglobin level remained unchanged and the serum iron concentration was persistently low it was concluded that the iron stores were depleted. If the hemoglobin concentration showed a significant rise within three to four weeks the phlebotomies were repeated until signs of depleted iron stores according to the above criteria were achieved. In some patients the performance of the complete phlebotomy program was not possible without temporary interruption and in one (J. E.) the phlebotomies could be made only once or twice monthly. The number and frequency of phlebotomies for each patient are shown in Fig. 1. Two patients were given iron in conjunction with the phlebotomy treatment. Patient B. C. was given iron sulphate orally for 6 months (300 mg/day), patient H. G. was given iron dextrine intravenously after each phlebotomy during a period of ten weeks. The amount of iron administered to the latter was equal to the calculated iron content of the removed blood and the total amount of iron dextrine given was 2700 mg. He was phlebotomized more frequently than the other patients (5–6 times monthly). Two patients (S. B. and A. G.) were given iron after a completed phlebotomy program.

The desferrioxamine test was performed initially in all patients and then at regular intervals. Porphyrin excretion in urine was determined before treatment and followed every week or every other week during treatment. Porphyrin excretion in faeces was determined before and after the accomplished course of phlebotomy treatment.

The patients were informed as to the probable connection between the disease and alcohol consumption. They were not "forbidden" to consume alcohol but told to report their alcohol consumption. Six patients did not alter their alcohol consumption, two (T. S., A. G.) stopped consuming alcohol entirely and the rest of the patients reduced their alcohol consumption more or less (Table I).

The chloropropamide and tolbutamid treatment in the two patients with diabetes mellitus and the hydantoin treatment in the patient with epilepsy were continued during the phlebotomy treatment.

No patient received any other form of treatment for

Table II. *Liver histopathology before treatment and liver function tests before and after phlebotomy treatment*

Pat.	Liver histology	Bilirubin/s (mg/100 ml) N.v. <1.1 Before	After	GOT/s (units) N.v. <40 Before	After	BSPR (retention) N.v. <5 Before	After	Glucose tolerance (k value) N.v. >1.0 Before	After
E. S.	Steatosis	0.5	0.4	48	29	16	7	0.75	1.31
H. P.	Fibrosis, periportal round cell infiltration	1.1	0.7	116	88	34	11	1.08	
S. B.	Steatosis	0.5	0.6	44	24	6	3	0.70	0.94
G. S.	Steatosis	1.4	0.7	60	39	19	11	0.83	1.25
G. Å.		1.4	1.2	85	45	25	10	1.07	
T. S.	Steatosis	1.0	0.4	44	40	10	6	1.31	
B. C.	Steatosis, fibrosis, periportal round cell infiltration	0.7	0.2	51	30	9	5	Overt diabetes	
H. G.	Steatosis	0.6	0.2	44	21	8	6	1.61	
J. K.	Steatosis, fibrosis, periportal round cell infiltr.	0.7	0.4	56	19	10	6	Overt diabetes	
A. G.		1.1	0.4	72	24	3	1	0.64	0.85
J. E.	Normal	0.3	0.4	40	43	10	9	0.82	1.17
M. I.	Normal	0.5	0.3	65	21	14	3	1.51	
Mean		0.8	0.5	60	35	14	7		
Mean difference		0.3		25		7			
S.E. of mean difference		0.1		4		2			

their porphyric disease. One patient has been hospitalized for many years because of epilepsy (J. E.) but no other patient was hospitalized longer than was necessary for the examinations (1–2 weeks). The treatment was generally started 1–2 weeks after the discharge from the hospital.

RESULTS

Laboratory Findings Before Phlebotomy

Porphyrin analyses (Table I)

All patients excreted large amounts of UP in the urine (2080–10 970 μg per 24 hours with a mean value of 4180) and the excretion of CP in the urine was usually moderately increased (110–530 μg per 24 hours, mean 315). The faecal excretion of CP and PP was also increased in nearly all patients and ranged from 8 to 352 (mean 59) and from 7 to 311 (mean 111) μg per g dry weight respectively. The urinary excretion of PBG and ALA was normal in all.

Liver histopathology and liver function tests (Table II)

Two of the ten patients in whom liver biopsy was performed had normal liver histology. The other eight showed varying degrees of steatosis and/or fibrosis. None had overt cirrhosis. Inflammatory changes were seen in three patients.

Serum bilirubin was borderline or above normal in five patients. The SGOT ranged between 37 and 116 units with a mean of 60 units. The BSPR was abnormal in all but one. The alkaline phosphatases and the prothrombin time were normal in all. Paper electrophoresis showed normal serum albumin values in all but one (J. E.) who also had an increased gamma globulin fraction and increased thymol turbidity.

In the patients without overt diabetes mellitus the intravenous glucose tolerance test showed decreased tolerance in five and normal in five.

Iron stores (Table III)

Signs of disturbed iron metabolism were seen in nearly all patients. The serum iron level was high in most subjects and the mean value was significantly higher than that of male controls. The TIBC level was within the range of normal except in one (J. E.). The mean desferrioxamine induced urinary iron excretion of the porphyric group was significantly higher than that of a control group comprising 25 healthy male sub-

Table III *Iron studies in serum and liver*

							Storage iron in the liver		
							Chemical determination of non hemin iron	Histochemical estimation of stainable iron (grade 0–4+)	
Pat	Sex	Hb (g/100 ml)	Fe/s (μg/100 ml)	TIBC (μg/100 ml)	TIBC saturation (%)	Fe/urine (mg/24 h)	(mg/100 g dry wt)	In parenchymal cells	In Kupffer cells
E S	♂	14 4	168	3?3	53	1 30	232	+ + +	+ + +
H P	♂	15 0	210	341	62	1 42	206	+ + +	+ + +
S B	♂	14 6	133	373	36	0 73	80	+ +	+ +
G S	♂	15 8	189	377	36	2 21		+ + +	+ + +
G Å	♂	16 7	156	268	58	2 28			
T S	♂	14 5	259	326	79	1 30	163	+ + +	+ +
B C	♂	15 4	207	269	76	2 60	128	+ + +	+ +
J K	♂	14 2	300	331	91	4 33	598	+ + + +	+ + +
H G	♂	14 8	247	357	69	0 64	62	+ +	+
A G	♀	13 6	206	284	78	1 89			
J E	♀	13 4	114	204	56	0 71	245	+ + +	+ + +
M I	♀	12 8	206	315	65	0 67		+ +	+ +
Mean ± S.E.			200 ± 15	314 ± 14	63 ± 5	1 67 ± 0 3	214 ± 60		
Normal men Mean ± S.E. (number)			133 ± 8 (25)	331 ± 9 (25)	41 ± 3 (25)	0 76 ± 0 04 (25)	97 ± 9 (18)		
Range			72–275	264–417	25–79	0 47–1 27	45–192		

jects. The urinary iron excretion was above the range of the control group in seven of the men and in one of the women.

Non hemin iron in liver biopsy specimens was determined in eight patients. Values above the normal range were found in four of them. The mean value was 214 mg per 100 g dry weight. The range (62–598 mg) was however wide. The mean value in a control group comprising 18 males was 97 mg with a range of 44–192 mg per 100 g dry weight.

Histochemically demonstrable iron was present in both parenchymal cells and Kupffer cells in all porphyric subjects studied. In *parenchymal liver cells* three had stainable iron of grade 2+ and six of grade 3+. Only one (J. K.) had stainable iron of the highest grade (4+). In *Kupffer cells one had stainable iron of grade 1+*, four 2+ and five grade 3+. In a control series comprising 20 normal men stainable iron in parenchymal liver cells was found in 15. Grade 1+ was found in eight, grade 2+ in five and grade 3+ in two subjects. In the control series stainable iron in Kupffer cells was demonstrated in only three subjects (two had grade 1+ and one grade 2+).

Effects of Phlebotomy Treatment

Two to 14 (mean 7) litres of blood were removed from patients who did not obtain iron supplements during phlebotomy treatment (Table IV). The mean period of treatment for the same patients was 4 3 months.

Clinical signs and porphyrin excretion

Phlebotomy treatment was associated with loss of active porphyric skin symptoms in all treated patients. Thus skin fragility, ulcers and blisters consistently disappeared during the treatment.

A marked decrease in porphyrin excretion accompanied the phlebotomy treatment in all patients (Table I, Figs. 1–3). The UP excretion in urine diminished to about 1/10 of the original amount. In one patient (H. P.) the UP excretion was normalized. In most patients the CP excretion in urine diminished and became normal. The faecal excretion of porphyrin diminished in all patients examined.

The clinical remission roughly coincided with the time when the urinary porphyrin excretion fell to about one mg per day (Fig. 2). With two exceptions (J. E., B. C.) this occurred 2–6 months

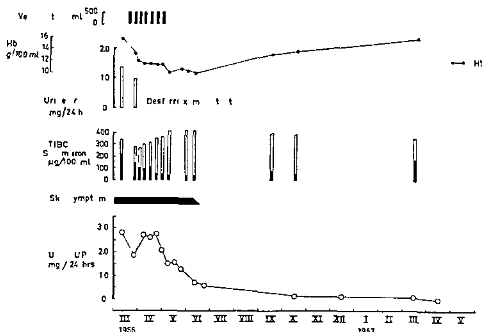


Fig 2 Biochemical changes during phlebotomy treatment in a 4? year old man (H P) with porphyria cutanea tarda

after the commencement of phlebotomy treatment Longer periods of treatment were required in the patient (J E) who could be phlebotomized only once or twice monthly and in the patient (B C) who was given iron orally in conjunction with phlebotomy treatment The last patient had the longest treatment (15 1/ months) and he did not improve until the iron administration was withdrawn and the iron stores were depleted In the patient (H G) who was given iron dextrin intravenously the porphyrin excretion diminished temporarily during intensive phlebotomy treatment Later however it increased continually in spite of the phlebotomies The iron administration was then stopped After one more month of phlebotomy treatment the UP excretion fell below 1 mg per day and he became free of symptoms

Table IV *Amount of removed blood treatment period and time required until the UP excretion in urine had fallen to one mg per day*

Pat	Blood removed (l)	Treatment period (mo)	Period with excretion in urine above 1 mg/day (mo)
E S	6 9	4½	4
H P	4 2	1½	2½
S B	4 2	3½	4½
G S	8 0	4½	3½
G Å	8 2	5	3
T S	10 .	6	3
B C	2? 6	15½	16
H G	11 0	5	4
J K	14 3	6½	6
A G	4 7	4½	3½
J E	6 9	8½	8½
M I	2 1	1½	2

Most patients tolerated the treatment well but some felt tired and had symptoms from the induced iron deficiency anaemia

During follow up periods of 6 to 18 (mean 10) months no relapse was seen in any of the patients who did not obtain iron after induced remission In one patient (A G) oral iron administration was started about eight months after induced remission (Fig 3) She was given 400 mg of iron sulphate daily for 30 days and then 300 mg daily for 15 days The urinary UP excretion showed increasing values after 3 1/ months and after two months more the UP excretion increased to about 1 5 mg per day and subsequently rose to about five mg per day She has now again been treated with phlebotomies and the UP excretion has diminished (Fig 3) Another patient (S B) was given 1100 mg iron dextrin intravenously

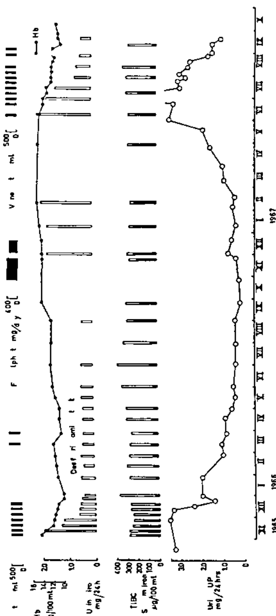


Fig 3 Biochemical changes during phlebotomy treatment and after oral iron administration in a 40 year old woman (A G) with porphyria cutanea tarda Note the effect of oral iron administration on the desferrioxamine induced urinary iron excretion and the excretion of uroporphyrin

eight to nine months after induced remission Increased UP excretion was noted after four months with a subsequent rise to about seven mg per day

Liver function tests and glucose tolerance (Table II)

The liver function tests were improved after treatment The serum bilirubin level was significantly lower and all had values below 0 8 mg per 100 ml after treatment Thymol turbidity serum electrophoresis prothrombin time and alkaline phosphatases were essentially unchanged The bromsulphalein retention diminished in all and became normal in two patients The SGOT diminished or became normal in nearly all patients It persisted on a high level after treatment in only one patient (H P) He probably had infectious hepatitis 15 years earlier

In one of the diabetic patients the tolbutamide dosage could be reduced from 0 5 g twice a day to 0 5 g on e a day without increase in the blood sugar level In the other patient with overt diabetes the chloropropamide dosage was unchanged during treatment In the five others with decreased glucose tolerance this was improved in all after phlebotomy treatment

DISCUSSION

Signs of disturbed iron metabolism in PCT have been reported High serum iron values are common findings (18) Histochemically visible iron in the liver seems to be present in most cases (3 34) Stainable iron in the liver was however a normal finding in a male control series In the individual case the histochemical estimation of stainable iron is an unreliable measure of the iron content of the liver (39) However the porphyric group in this study had more stainable iron than a control series They differed strikingly from the control series with regard to stainable iron in Kupffer cells Furthermore four out of eight porphyric patients examined had a non hemin iron content in the liver which was above the normal range None had a liver iron concentration of the magnitude encountered in fully developed idiopathic hemochromatosis Increased desferrioxamine induced iron excretion was found in eight out of twelve patients

Increased iron stores in PCT has been thought

to be due to increased intake of iron in alcoholic beverages (18) A number of wines contain significant quantities of iron Furthermore some recent experiments show that alcohol increases the absorption of ferric iron (5) In this series however only one patient was a wine drinker The woman (J E) who was a teetotaler had a high liver iron concentration Moreover in a series of advanced chronic drinkers of distilled spirits we rather found decreased iron stores (21) Hence we consider that the increased iron stores in PCT in this series was unrelated to alcohol consumption Increased absorption of food iron or faulty release of iron is more probable

The phlebotomy treatment was associated with loss of active porphyric skin symptoms in all patients and the porphyrin excretion in urine decreased to about one tenth of the original amount Spontaneous remissions occur and in alcoholics who stop drinking the porphyrin excretion may become normal (35) It is possible that some of the patients in this series improved because of diminished alcohol consumption Most of our patients however continued to consume alcohol and in six of them alcohol consumption was unchanged The course was the same and all improved Not only did disappearance of skin symptoms and decreased porphyrin excretion accompany the phlebotomy treatment but the liver function tests also improved in all The improved tolerance was perhaps also due to im liver function The favourable effect on liver function tests was also noted in the patients with unchanged alcohol consumption

The way in which phlebotomy treatment affects the porphyrin metabolism in PCT is not clear Ippen (15) who introduced the phlebotomy treatment in this disease has put forward the theory that the pathological porphyrins and their precursors take part in the compensatory formation of new hemoglobin He does not believe in a metabolic block in hemoglobin synthesis since anaemia is not a feature of the disease However the existence of an enzymatic block for hem synthesis located solely in hepatic cells might be considered Such a hepatic block might explain the accumulation of porphyrins and would not necessarily imply defective hemoglobin production in the bone marrow

The effect of the phlebotomy treatment may be related to the reduction of iron stores In favour of this view is the fact that the UP excretion remained at a high level in the two patients who were given iron in conjunction with phlebotomy treatment Furthermore the only patients who had relapses were those who were given iron after induced remissions The last argument must however be evaluated with caution as only two of the other patients had observation periods of the same length as the two patients given iron after remission Signs of increased iron stores were present in most of our patients However some who had normal liver iron concentrations also improved and it is probable that even normal amounts of iron in the liver cells may have a noxious influence on the porphyrin metabolism in porphyric individuals As PCT is uncommon in iron storage disease it is however probable that disturbed iron metabolism is not the primary cause of the disease

The improved liver function tests after treatment may imply that the accumulation of porphyrins and iron in the liver cells may have a noxious effect on liver cell function Therefore it seems indicated to phlebotomize also patients with latent PCT The frequent finding of hepatic carcinoma in the autopsy series of Braun and Berman (4) favours this view

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REFERENCES

1 Askevold R Routine analysis of porphyrines in urine Scand J clin Lab Invest 3 318 1951
2 Babson A L Shapiro P O Williams P A R & Philips G E The use of a diazonium salt for the determination of glutamic-oxalacetic transaminase in serum Clin chim Acta 7 199 1962
3 Berlin S O & Brante G Iron metabolism in porphyria and haemochromatosis Lancet ? 7?9 196
4 Braun A & Berman J Pathological anatomy in porphyria cutanea tarda Acta Univ Carol Med (Praha) 5 597 1959
5 Charlton, R. W Jacobs P Seftel H & Bothwell T H Effect of alcohol on iron absorption Brit. med J 2 1427 1964
6 Epstein, J H & Redeker A G Porphyria cutanea tarda symptomatica A study of the effect of phlebotomy therapy Arch Derm. 9? ?86 1965
7 Felsher B F & Redeker A G Effect of chloroquine on hepatic uroporphyrin metabolism in patients with porphyria cutanea tarda Medicine (Baltimore) 45 575 1966

8 Gajdos A & Gajdos-Torok M Studies on the porphyrias in France S Afr J Lab clin Med 9 295 1963
9 Hæger Aronsen B Various types of porphyria in Sweden. S Afr J Lab clin Med 9 288 1963
10 Holmes, J G & Barnes H D Cutaneous porphyria in alcoholic siblings Trans St John s Hosp derm Soc (Lond) 51 55 1965
11 Holti, G Rimington C Tate B C & Thomas G An investigation of porphyria cutanea tarda Quart J Med 27 1 1958
12. Hutchison, H E The significance of stainable iron in sternal marrow sections Blood 8 236 1953
13 Ikkos D & Luft, R. Intravenos glykosbelastning Nord Med 57 134 1957
14 Ippen, H Entstehung und Behandlung der Porphyria cutanea tarda. Klin Wschr 38 89 1960
15 — Zur Patho enese der Porphyria cutanea tarda Arch klin exp Derm 212 467 1961
16 — Beobachtungen bei der Aderlassbehandlung der Porphyria cutanea tarda Arch klin. exp Derm 213 863 1961
17 Koch J & Pirnsch K. Einige klinische und therapeutische Aspekte zur Porphyria cutanea tarda Dtsch Gesundh Wes 0 156 1965
18 Kramer S Iron metabolism in the porphyrias S Afr J Lab cl n Med 9 .83 1963
19 Lundin, P., Persson E & Weinfeld, A. Comparison of hemosiderin estimation in bone marrow sections and bone marrow smears Acta med scand 175 383 1964
.0 Lundvall O & Weinfeld A Determination of iron in urine with special reference to the desferrioxamine test. J Clin Path 0 611 1967
21 Lundvall O., Weinfeld, A & Lundin, P Iron stores in alcohol abusers To be published
?2. MacLagan N F Thymol turbidity test, new indicator of liver dysfunction Brit J exper Path. 25 .34 1944
23 Mauzerall D & Granick, S The occurrence and determination of delta amino-levulinic acid and porphobilinogen in urine J b ol Chem. 219 435 1956
24 Peters H A., Johnson S A. M., Cam S Oral S Muftu, Y & Ergene T. Hexachlorobenzene-induced porphyria Effect of chelation on the disease porphyrin and metal metabolism. Amer J med Sci .51 314 1966
25 Peters T Govanello T J Apt L & Rose J F A new method for the determination of serum iron b nd ng capacity J Lab clin Med 48 274 1956
6 Rimington C & S einsson, S L The spectrophotometric determination of uroporphyrin Scand J clin Lab Invest. .. .09 1950
27 Roos, K. Automated procedure for simu taneous or separate determination of bilirubin and alkaline phosphatase in serum. Scand J clin Lab In est. 17 197 1965
.8 Rosenthal, S M & Wh te E C Clinical application of the bromsulphalein test for hepatic function J Amer med Ass 84 111., 19 5
9 Schaerstrom, R. & Lundvall O Porphyria cutanea tarda och hemosideros Nord Med 7.. 143., 1964
30 Schirren, C Strohmeyer G Wehrman, R. & Wiskemann A. Ergebnisse der Aderlassbehandlun., bei Porphyria cutanea tarda Dtsch med. Wschr 91 1344 1966
31 Stathers G M Porphyrin-binding effect of cholestyramine Lancet 2 780 1966
3. Streda, M & Berman J Zum Kohlenhydratstoffwechsel bei Porphyria cutanea tarda. Z. ges inn Med 20 90 1965
33 Sweeney G D., Saunders S J Dowdle E B & Eales, L Effects of chloroquine on patients with cutaneous porphyria of the symptomatic" type Brit med J 1 1 81 1965
34 Uys C. J & Eales L. The histopathology of the liver in acquired (symptomatic) porphyria S Afr J Lab clin Med 9 190 1963
35 Waldenstrom, J & Haeger B The liver in porphyria cutanea tarda Ann. intern Med. 53 286 1960
36 Watson C J Pimenta de Mello R., Schwartz, S Hawkinson, V E & Bossenmeier I Porphyrin chromo ens or precursors in urine blood bile and feces J lab clin Med 37 831 1951
37 Watson, C J The problem of porphyria — some facts and questions New Engl J Med 263 1 05 1960
38 Weinfeld, A Storage iron in man. Acta med scand Suppl 4.7 13 1965
39 Weinfeld, A., Lundin P & Lundvall O Si.nificance for the diagnosis of iron overload of histochemical and chemi al iron in the liver of control subjects J clin. Path 21 35 1968
40 With T K Porphyrin concentration from ultraviolet extinction A note on the calculations Scand J clin lab Invest. 7 193 1955
41 Zimmerman, T S McMillin, J M & Watson C J Onset of manifestations of hepatic porphyria in relation to the influence of female sex hormones Arch intern Med 118 2.9 1966

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COMPARATIVE STUDIES ON INTRAMUSCULAR AND ORAL EFFECTIVE DOSES OF SOME ANTICHOLINERGIC DRUGS

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Abstract Sixteen healthy volunteers received propantheline methylscopolamine or butylscopolamine in oral as well as i m administration After propantheline and methylscopolamine salivary secretion was found to be more influenced than the heart rate and the near point of accommodation The relations between i m and oral doses producing comparable total effects on salivation were 1 10 for propantheline and 1 100 for methyl scopolamine After oral butylscopolamine in doses up to 480 mg no anticholinergic effects were observed In contrast to the other compounds i m butylscopolamine produced only transient effects and influenced mainly the near point of accommodation It is concluded that presumably less than 10 per cent of methylscopolamine and probably only very little of butylscopolamine is absorbed in the gastrointestinal tract

The ratio between parenteral and oral effective doses of drugs reflects some of their pharmacological properties among which the gastrointestinal absorption may be the most important Although such a determination is relatively simple to perform published observations of this kind are few

In this investigation effective intramuscular and oral doses of propantheline methyl and butyl scopolamine were studied in man on the salivary secretion the heart rate and the accommodation of the eye The responses to the two different administrations of each drug were compared quantitatively in the same subjects

MATERIAL AND METHODS

Fifty-one experiments were performed using 16 healthy volunteers aged between 0 and 35 comprising nine females and seven males The salivary secretion the heart rate and the near point of accommodation were studied in the following manner The salivary secretion was stimulated by having the subject chew a tablet of ascorbic acid (250 mg) during 30 sec The saliva obtained including that produced during the following 30 sec was spat out and the total volume was measured as previously described (11) In this paper the term salivary secretion refers only to saliva obtained in this manner The heart rate was taken as being that of the frequency of the radial pulse The near point of accommodation was determined as the shortest distance at which the subject was able to read very fine print with one eye In this test each subject consistently used the same eye

The drugs were administered after the subjects had rested in a sitting position for 40 min During this time three determinations of the three parameters were made at intervals of 15 min At the beginning of the investigation it was noted that the first of the three determinations of salivary secretion constantly showed a lower value than the following two and so this value was excluded At the end of the investigation when the intramuscular route was used with the same subjects all three control values were similar

Oral administration was in the form of commercially available tablets The parameters were continuously determined at hourly intervals during the following eight hours Placebo tablets containing lactose were also given The experiments were carried out with a double blind technique

The injections were given i m in the thigh The parameters were determined at intervals of 15 min during the first hour at intervals of 30 min during the second hour and at hourly intervals subsequently up to and including the eighth hour after the administration of the drug

The subjects were either fasting or had had a light breakfast about two hours before the experiment They usually had a light lunch three or four hours after the drugs had been given The drugs studied the doses used the number of experiments and the compound each subject received are shown in Table I One subject received atropine The effects of oral administration of propantheline and methylscopolamine were studied in 11 subjects The responses to intramuscular administration were studied on four of these subjects

Quantitative comparisons between the effects of intramuscular and oral administration of the drugs were carried out as follows The mean of each parameter for the predrug estimations was drawn as a continuous baseline

Table I *Participating subjects on each drug route of administration and dose*

Drug	Oral dose (mg)	Subjects	Intramuscular dose (mg)	Subjects
Placebo (lactose)		RS HB IW PH EK		
Atropine sulphate	2	IJ	0 33	IJ
(Atropin ACO)	4	IJ	1 0	IJ
			3 0	IJ
Propantheline bromide	30	CH BK EK PH	3	CH BK
(Pro Banthine Searle)	60	CH BK EK PH	10	CH BK
	120	CH BK EK PH		
Methylscopolamine nitrate	4	TM IS RT BMC EW	0 16	CO JM
(Skopyl Pharmacia)	8	CO JM RT BMC	0 50	CO JM
	16	CO JM RT BMC		
Butylscopolamine bromide	240	IW KÖ	10	IW KÖ
(Buscopan Boehringer/ Ingelheim)	480	IW KÖ	30	IW KÖ

on a diagram The hourly post administration values which formed an effect curve were entered on this diagram The area between the baseline and the effect curve was measured planimetrically for each subject parameter and dose These areas were then compared

Student s *t* test was used when a statistical analysis of the material was performed

RESULTS

The *placebo* group had a constant response to stimulation of salivary secretion during the eight hours of the experiment (Fig 1) The heart rate showed no noteworthy variations with the exception of a slight increase at the determination that followed lunch (the fourth hour) The near point of accommodation was increased in some of the subjects at the last two determinations

Atropine 2 mg orally caused a moderate inhibition of the salivary secretion within one hour a 4 mg oral dose produced a greater inhibition at this time However during the period of eight hours there was no significant difference between the total effects of the two doses (Fig 2 *a*) Intramuscular administration of 3 mg of atropine caused a corresponding degree of inhibition of salivary secretion during the eight hour period (Fig 2 *b*) During the same period of observation the effect on heart rate of 4 mg of atropine orally was approximately equal to that of 1 mg intra

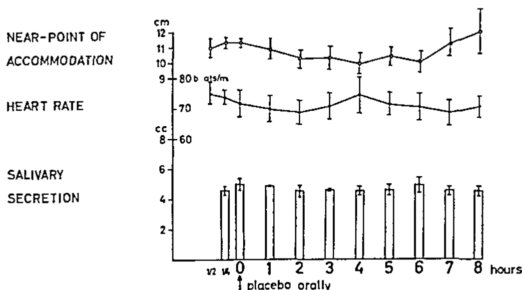


Fig 1 The influence of oral placebo on three cholinergic functions in five subjects (means ± S E.)

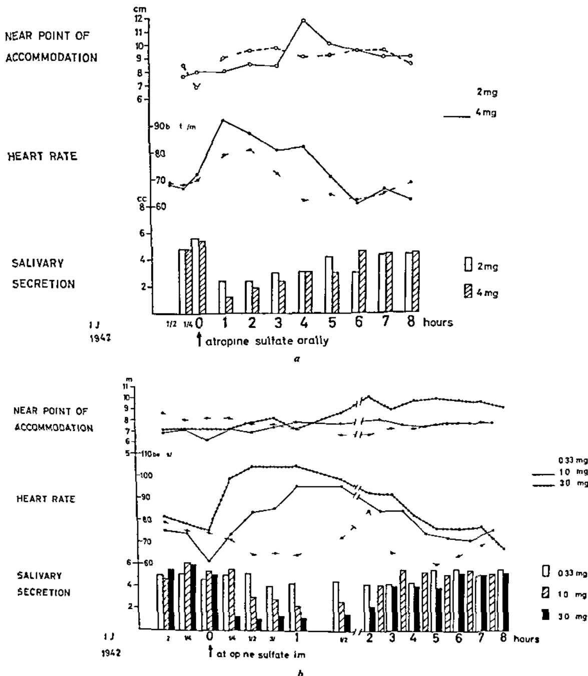


Fig 2 (*a*) Anticholinergic effects of atropine 2 and 4 mg orally in subject I J (*b*) Anticholinergic effects of atropine 0 33 1 0 and 3 0 mg i m in subject I J

muscularly The near point of accommodation tended to increase after the largest doses of atropine (Fig 2 *a* and *b*)

Propantheline 30 mg orally caused a slight decrease in the salivary secretion Sixty mg and 120 mg resulted in a substantial inhibition of the secretion ($p < 0.01$) and at the higher dose in tachycardia as well ($p < 0.05$) (Fig 3) No effect on the near point of accommodation was observed in two subjects while in the remaining two the

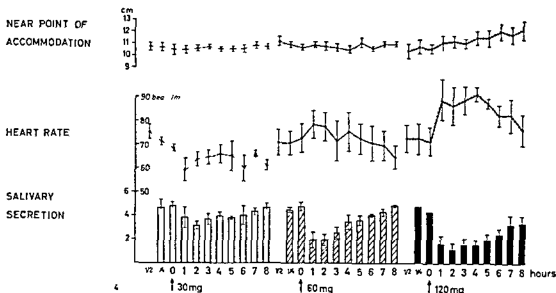


Fig 3 Anticholinergic effects of propantheline 30 60 and 120 mg orally in four subjects (means ± S E)

distance increased moderately after the highest dose

Thirty mg of propantheline orally depressed salivation during eight hours approximately as much as 3 mg intramuscularly in the same subjects The effect of 60 mg orally corresponded rather well to that of 10 mg intramuscularly The results from one of the two subjects are shown in Fig 4 *a* and *b* The other subject showed similar results Propantheline 120 mg orally increased the heart rate in both subjects to the same degree as 10 mg intramuscularly

Methylscopolamine 16 mg orally caused an inhibition of salivation ($p < 0.05$) and a tachycardia (Fig 5) It was found that the effect on salivation obtained with this dose corresponded quantitatively to that of 0 16 mg administered intramuscularly (Fig 6 *a* and *b*) In one subject the increase in the heart rate following 16 mg of methylscopolamine orally was somewhat less than that of 0 50 mg intramuscularly (Fig 6 *a* and *b*) In the other subject 16 mg orally increased the heart rate half as much as 0 50 mg intramuscularly At the doses used neither oral nor parenteral administration had any effect on the near point of accommodation

Butylscopolamine in the doses of 240 and 480 mg orally and 10 mg intramuscularly did not influence salivation heart rate or the near point of accommodation The results from one of the subjects are shown in Fig 7 *a* and *b* Thirty mg intramuscularly caused in this subject a slight inhibition af salivary secretion an increase in heart rate and a very pronounced increase in the near point of accommodation In the other subject the effects were quantitatively less In both subjects the effects were of very short duration.

Symptoms None of the subjects in the placebo group experienced any symptoms The others felt dryness of the mouth simultaneously with demonstrable inhibition of salivation In two subjects this feeling was reported without measurable salivary inhibition When the decrease of the salivary secretion was pronounced the subjects also complained of roughness or slight pain in the throat One subject reported tired eyelids after 16 mg of methylscopolamine orally while another complained of headache after 0 5 mg intramuscularly One subject became drowsy after 10 mg of propantheline another after 10 mg of butylscopolamine intramuscularly

DISCUSSION

As the results are based on repeated measurements of three parameters during more than eight hours it was considered important to evaluate the possible appearance of other than drug induced fluctuations in the parameters In the placebo studies no such significant variations were ob-

NEAR-POINT OF ACCOMMODATION (cm)

HEART RATE (beats/min)

SALIVARY SECRETION (cc)

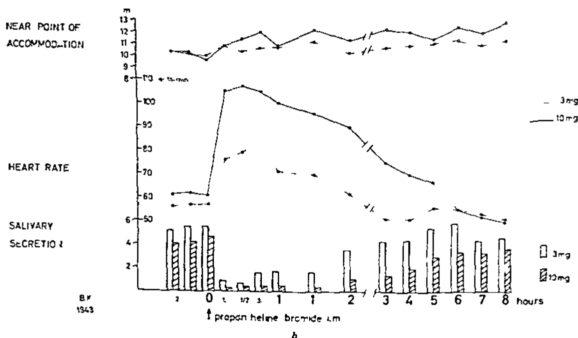
30 mg — 60 mg — 120 mg

B K 1943

0 1 2 3 4 5 6 7 8 hours

↑ propantheline bromide orally

a



b

Fig. 4. (a) Anticholinergic effects of propantheline 30, 60 and 120 mg orally in subject B. K. (b) Anticholinergic effects of propantheline 3 and 30 mg i.m. in subject B. K.

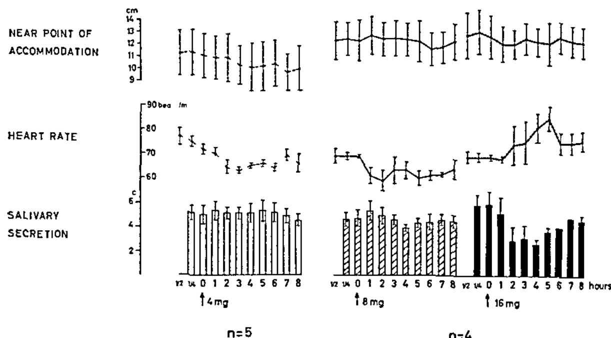


Fig 5 Anticholinergic effects of methylscopolamine 4 8 and 16 mg orally in four subjects (means ± s e)

served apart from a moderate increase in heart rate in connection with lunch and a mild decrease in the accommodation of the eye during the seventh and eighth hour possibly due to fatigue

For further evaluation of the method the effects of atropine were studied and found to be in accordance with earlier observations (2) and with the known fact that atropine is well absorbed from the gastrointestinal tract

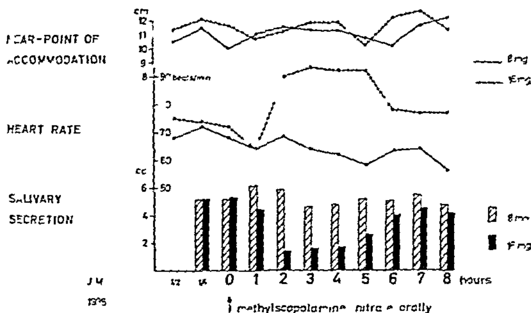
The ratio between the effective intramuscular and oral doses of propantheline was found to be approximately 1 10 There seem to be no earlier published studies comparing the effects of parenteral and oral propantheline in the same subject The effective oral dose is similar to that previously observed by other authors describing effects on the salivary secretion (14) and the gastric acidity (6 8 10 13)

The ratio between intramuscular and oral effective doses of methylscopolamine on salivation was found to be approximately 1 100 The minimum oral dose of methylscopolamine required to produce any effect on the studied parameters was about 8 mg As 0 16 mg intramuscularly produced extensive effects lasting a couple of hours it is likely that less than 10 per cent of the dose administered orally was absorbed Levine (9) showed that 10 to 30 per cent of methylscopolamine is absorbed from ligated intestinal loops in rats

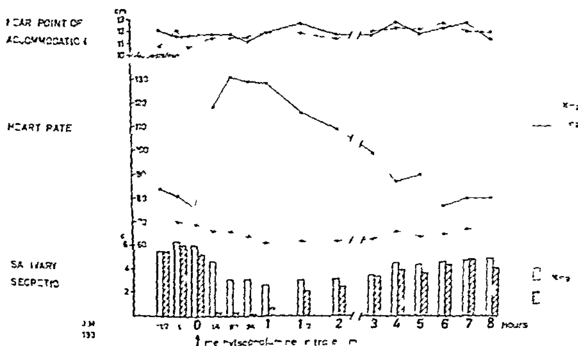
Investigations of cholinergic functions other than those studied here have also given results that indicate an incomplete absorption of methylscopolamine in the gastrointestinal tract The ratio between the parenteral and oral effective dose of the compound on gastric secretion has been found to be 1 500 (6 7) These authors have used the bromide salt of the compound while the nitrate was used in the present study

The effects of parenteral butylscopolamine were found to differ both qualitatively and quantitatively from those caused by the other drugs Firstly butylscopolamine caused effects of remarkably short duration Secondly its effect on the near point of accommodation was extensive in a dose which produced only a moderate effect on salivary secretion The other compounds were distinguished by more protracted effects and by a more extensive effect on the salivary secretion than on the other parameters studied Such effects are considered to characterize anticholinergic drugs (4 12)

The brief effect of butylscopolamine indicates



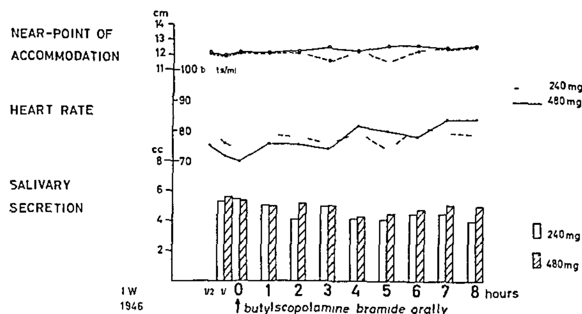
a



b

Fig 6 (*a*) Anticholinergic effects of methylscopolamine 8 and 16 mg oral in subject J M (*b*) Anticholinergic effects of methylscopolamine 0 16 and 0.0 mg i.m. in subject J M

a rapid inactivation and or a rapid excretion. Herxheimer and Haefeli (5) estimated this to be about 20 mg per hour for a 70 kg adult, which is far from sufficient to explain the ineffectiveness of oral administration of 480 mg. Probably only a very small amount of administered butylscopolamine is absorbed in the gastrointestinal tract as has previously been pointed out (3 5) The ineffectiveness of oral butylscopolamine has also been demonstrated on gastric acidity osmolarity



a

b

Fig 7 (*a*) The influence of butylscopolamine 240 and 480 mg orally on three cholinergic fun tions in subject I W (*b*) Th influence of butyl copolamine 10 and 30 mg i m on three cholinergic functions in subject I W

and gastric evacuation rate Thus 480 mg produced only slight effects on these parameters while small amounts of oral atropine and propantheline elicited extensive anticholinergic responses (1)

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REFERENCES

1 Bromster D Carlberger G Lundh G Moller J & Rosén A The influence of some oral anticholinergics on gastric emptying and osmolarity To be publ shed

2 Cullumbine H McKee W H E & Creasey N H The effects of atropine sulphate upon healthy male subjects Quart J exp Physiol 40 309 1955

3 Drug Therap Bull 1 39 1963

4 Herxheimer A A comparison of some atropine like drugs in man with particular reference to their end organ specificity Brit J Pharmacol 13 184 1958

5 Herxheimer A & Haefeli L Human pharmacology of hyoscine butylbromide Lancet ? 418 1966

6 Kirsner J B & Palmer W L Newer gastric anti secretory compounds Amer med Ass J 151 798 1953

7 Kirsner J B Levin E & Palmer W L Pamine bromide Gastric antisecretory effects and thera peutic usefulness in peptic ulcer and other gastro intestinal disorders Gastroenterology 26 852, 1954

8 Kirsner J B Ford H & Kassriel R S Anti cholinergic drugs in peptic ulcer Med Clin N Amer 41 495 1957

9 Levine Mitchell R The intestinal absorption of the quaternary derivates of atropine and scopolamine Arch int Pharmacodyn 121 146 1959

10 McKenna R D Bourne P H & Arendt E A comparative study of three anticholinergic drugs — Monodral Pamine and Pro-Banthine Canad med Ass J 74 685 1956

11 Moller J & Rosén A Pharmacological studies of the effectiveness of anticholinergic drugs adm nistered perorally Lakartidn Suppl 2 63 1967

12 Nyman, E Studien uber die Atrop ngruppe Acta physiol scand Suppl 10 1942

13 Sun D C H & Shay H Optimal effective dose of ant cholinergic drugs in peptic ulcer treatment Arch intern Med. 97 447 1956

14 Zupko A G & Prokop L D The newer anti cholinergic agents II Effectiveness as antisialogogues J Amer pharm Ass 43 219 1954

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ON THE PREVALENCE OF ADRENOCORTICAL ADENOMAS IN AN AUTOPSY MATERIAL IN RELATION TO HYPERTENSION AND DIABETES

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Abst act A prospective study was performed on an autopsy material consisting of 739 consecutive cases over 20 years of age (391 females 348 males) during a period of six months The material included about 70% of the deaths in the city of Malmo (approximately 250 000 in habitants) The adrenals were specially observed at autopsy for adenomas and hospital records were searched for hypertension and diabetes mellitus

In the total material the frequency of adrenocortical adenomas was 8 7% which was cons derably higher than previously reported figures In patients with essential hypertension the adenoma frequency was 12 4% as against 7 9 in normotensives This difference was not statistically s gnificant. The adenoma frequency in men with secondary hypertension was remarkably high but the significance is not clear In patients with hypertension the frequency of d abetes was 17 6% as compared to 9 0% in normotensives Th s difference was statistically probably sig n ficant.

In patients with diabetes the frequency of adrenocortical adenomas was 16 5% in non-diabetics 7 7 The dif ference was probably significant

The syndrome of primary aldosteronism described by Conn (1) in 1955 is characterized by hyperten sion hypokalaemia and inappropriately increased production of aldosterone from an autonomous adrenocortical adenoma Reports on verified cases with this syndrome show that these patients as a rule have had moderate hypertension with limited clinical manifestations for a long period of time whereas symptoms and signs of hypokalaemia have appeared rather late The hypokalaemic symptoms often seem to have been the essential guide to correct diagnoses in most cases (5) The recogni tion of this fact has brought up the question of frequency of primary aldosteronism amongst pa tients with hypertension and it has been asked whether a significant number of patients with so-called essential hypertension in reality suffer from primary aldosteronism with normokalaemia Conn has advanced the hypothesis that 15–20% of all patients with benign essential hypertension have primary aldosteronism (3 4) He arrived at these figures on the basis of earlier reports showing a higher frequency of adrenal adenomas in patients with hypertension (8 9) as compared to normals Furthermore Conn assumed that the frequent presence of decreased glucose tolerance amongst hypertensives (7) is caused by primary aldosteron ism decreased glucose tolerance being found in about 50 of all cases (2) The present investiga tion was concerned with the prevalence of adreno-cortical adenomas in relation to hypertension and diabetes mellitus in autopsy material from a random adult population

MATERIAL AND METHODS

The stud es were planned prospectively and included the autopsy material of Malmo General Hospital for six months February–July 1966 The material consisted of 739 patients, representing about 70% of the deaths within the city (total number of inhabitants about 250 000) dur ing that period Patients under the age of 20 were ex cluded but otherwise the material was unselected The sex and age distribution is presented in Fig 1

Autopsies were performed according to the routine at the department of pathology The adrenals were cut in mm wide slides and the presence of adenomas recorded The diagnosis was mainly on macroscopic criteria Ade nomas varied from 2 mm to 4 cm in diameter So-called nodular cortical hyperplas a was not included The ade nomas were studied microscopically (G Ö) and showed the same types of cells as the surrounding cortex

In all 739 cases the hosp tal records were investigated (H H) with respect to the presence of hypertension or diabetes mellitus A diagnosis of hypertens on was based on repeated readings of a d astolic blood pressure above 100 mm Hg and electrocardiographic or roentgenologic

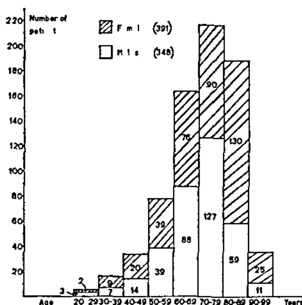


Fig 1 Sex and age distribution of the total autopsy material (739 patients)

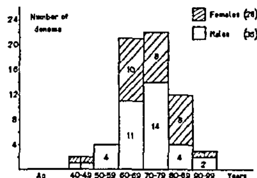


Fig 2 Distribution of adrenocortical adenomas according to sex and age in patients autopsied (391 females and 348 males)

signs of enlargement of the left ventricle or hypertrophy of the left ventricle at autopsy Hypertension was divided into probably essential hypertension and so-called secondary hypertension, where there was evidence of renal disease A diagnosis of diabetes mellitus was based on the presence of glycosuria and hyperglycemia > 1 0 mg%. The total number of diabetics was 79 in 76 the disease had presented after the age of 40 They had been treated with diet alone or in combination with sulphonyl urea or insulin

RESULTS

Sixty four cortical adenomas were found (Fig 2) representing a prevalence of 8 7 %. In the females the prevalence was 7 2 % and in the males 10 3 % (Table I)

Hypertension was found in 119 patients and 620 were normotensive In hypertensives the adenoma frequency was 12 4 % as compared to 7 9 % in the normotensives This difference was not statistically significant ($p > 0\,2$)

In women with essential hypertension the adenoma frequency was 7 7 % as compared to 7 4 % in normotensives (Table I) Thus there was no positive correlation between the presence of hypertension and adrenocortical adenomas in women In women with secondary hypertension no adenoma was found In men with essential hypertension the adenoma frequency was 18 9 % and 8 4 % in normotensives the difference is probably significant ($0\,05 > p > 0\,01$) In men with secondary hypertension the adenoma frequency was 26 7 % which gave a clear statistical difference against normotensives (Table I)

Diabetes was present in 17 6 % in hypertensives as compared to 9 0 % in normotensive patients The difference was probably significant p being close to 0 01

The adenoma frequency in diabetics was 16 5 % in non-diabetics 7 7 % This difference was probably significant ($0\,05 > p > 0\,01$) In female diabetics the adenoma frequency was 16 3 % in non diabetics 5 8 % ($0\,05 > p > 0\,01$) In male diabetics the adenoma frequency was 16 7 % in non

Table I *Frequency of adrenocortical adenomas in relation to hypertension in 739 consecutive autopsies*

Patients	391 Females			348 Males		
	With adenoma	Without adenoma	Frequency (%)	With adenoma	Without adenoma	Frequency (%)
Normotension	24	300	7 4	25	271	8 4
Ess. hypertension	4	48	7 7	7	30	18 9
Sec hypertension	0	15	0	4	11	26 7
Total number	28	363	7 2	36	312	10 3

Table II *Frequency of adrenocortical adenomas in relation to diabetes and hypertension in 739 consecutive autopsies*

	391 Females			348 Males		
Patients	With adenoma	Without adenoma	Frequency ( )	With adenoma	Without adenoma	Frequency ( )
No diabetes	20	322	5 8	31	287	9 7
Diabetes total number	8	41	16 3	5	25	16 7
Ess hypertension + diabetes	1	12	7 7	1	2	33 3
Normotension + diabetes	7	23	23 3	3	23	11 5
Sec hypertension + diabetes	0	6	0	1	0	(100)

diabetics 9 7% $(0\ 2 > p > 0\ 1)$ Thus there was a probably significant difference in adenoma frequency in females but not in males

The combination of adrenocortical adenoma essential hypertension and diabetes was found in only two cases giving a prevalence of 0 27% in the total material of 739 patients and 2 2% of all patients with essential hypertension Adenoma in combination with secondary hypertension and diabetes was found in one case

DISCUSSION

The frequency of adrenocortical adenomas in the present material was 8 7 % This figure is far higher than has been reported in some earlier investigations which showed a frequency of 1 4 (6 8) These earlier investigations were retrospective studies whereas the present was prospective and it seems probable that a prospective study principally leads to a higher degree of accuracy However the frequency of adrenocortical adenomas in the Malmö autopsy material for the period 1957–1965 varied between 5 0 and 9 5% from one year to the other according to routine observations and records There was no clear sex difference for that period and the adenoma predominance observed in men in the present material may be due to the rather limited number of cases

Some earlier reports give a remarkably high frequency of adrenocortical adenomas in hypertensive patients Thus Shamma et al (9) found that the adenoma frequency was ten times higher in hypertensives as compared to normotensives and Russi et al (8) found a fivefold increase However in a recent report no such difference was found (6) Our figures tend to support the latter findings In a study of the kind presented here the results are influenced by the criteria used to define hypertension In the report by Russi et al (8) the diagnosis of hypertension was based on heart weight exceeding 350 g and absence of myocardial or valvular disease Heart configuration was not taken into account nor were the case histories investigated with respect to hypertension In other studies (6 9) similar criteria were used although clinical records were taken into account to some extent We used more strict criteria in order to include only clinically significant hypertension (see above)

The high adenoma frequency in men with secondary hypertension and advanced renal damage is noteworthy Even though the significance of this finding is not clear a causal relationship is possible

Our investigations hardly support the hypothesis advanced by Conn that primary aldosteronism could be present in about 15–20% of all cases with so-called essential hypertension In our material the adenoma incidence was 12 4% in patients with essential hypertension (both sexes included) and this figure thus represents the highest possible relation of primary aldosteronism in essential hypertension This figure should however be evaluated in view of the adenoma frequency found in normotensives namely 7 9 From this one might conclude that primary aldosteronism can be of importance in at most 4 5% of cases with clinically significant hypertension However we do not know whether the adenomas were aldosterone producing either in the cases classified as hypertensives or in the cases classified as normotensives Thus the real role of the adenomas found in hypertensive patients cannot be established The macro and microscopic appearance of these

adenomas—whether found in hypertensives or normotensives—did not differ from that of aldosteronomas published earlier

Ostrander (7) observed an increased incidence of diabetes mellitus in patients with essential hypertension This was to some extent supported by the present investigation We found a high frequency of adrenocortical adenomas in the diabetic patients but the significance cannot be evaluated

ACKNOWLEDGEMENT

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REFERENCES

1 Conn, J W., Primary aldosteronism, a new clinical syndrome J Lab clin. Med. 45 3 1955
2. — Hypertension The potassium ion and impaired carbohydrate tolerance New Engl. J Med 273 1135 1965
3 Conn, J W., Cohen, E L., Rovner D R. & Nesbit, R. M Normokalemic primary aldosteronism. J Amer med Ass 193 200 1965
4 Conn, J W., Rovner D R., Cohen, E L. & Nesbit, R. M Normokalemic primary aldosteronism. J Amer med. Ass. 195 21 1966
5 Hokfelt, B Der primare Aldosteronismus Verh. dtsch. Ges inn. Med 68 616 196–
6 Kokko J P., Brown, T C & Berman, M M Adrenal adenoma and hypertension. Lancet 1 468 1967
7 Ostrander L D., Francis T J., Hayner N S., Kjelsberg, M O & Epstein F H.. The relationship of cardiovascular disease to hyperglycemia. Ann. intern. Med 62 1188 1965
8 Russi, S., Blumenthal H T & Gray S H Small adenomas of the adrenal cortex in hypertension and diabetes. Arch. intern. Med. 76 284 1945
9 Shamma, A. H., Goddard, J W & Sommers S C A study of the adrenal status in hypertension J chron. Dis 8 587 1958

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TISSUE AND PLASMA CORTISOL IN MAN UNDER VARIOUS CONDITIONS

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Abstract A recently developed micro-method for determination of cortisol in punch biopsy skin samples was applied to 158 patients with various diseases and drug treatments Measurements of the total plasma concentration of cortisol were made simultaneously The results seemed to reflect the actual cortisol content in the skin fairly accurately but considerable individual variations were present.

Prominent findings were the following Treatment with oestrogen (as oral contraceptives) increased the plasma cortisol 2 / fold with no concomitant change in the tissue concentration of cortisol Women in the years around the menopausal age were frequently found to have a low tissue cortisol concentration with a normal plasma cortisol Patients with connective tissue diseases including rheumatoid arthritis showed a non-significant elevation of the tissue cortisol but when such patients were treated with indomethacin the concentration of tissue cortisol fell significantly In approximately 10 of all the patients the tissue plasma ratio exceeded one Three of these cases were treated with chloroquine A moderate degree of positive correlation between plasma and tissue cortisol could only be demonstrated if factors believed to influence the distribution pattern of cortisol were avoided On the basis of these observations it is suggested that other mechanisms, in addition to a shift in the proportion of cortisol binding to plasma proteins affect the cortisol distribution in the body Protein binding within the tissue is discussed as a possibility

Except for the adrenal cortex precise information about the concentration of cortisol in tissues from human beings does not seem to be available However the relationship between plasma and tissue concentrations of cortisol in patients with various diseases hormonal disorders and in different drug treatments are of interest as shifts in free and bound forms of plasma cortisol may not be the only mechanisms by which this hormone in the tissues could be influenced A chemical rather than a radioisotopic technique should be preferred when dealing with human beings although static observations may have limited interpretations

The present study deals with the problem outlined above by using a recently developed micromethod (6) by which cortisol is spectrophotofluorometrically determined in small tissue samples obtained by punch biopsies General information concerning the usefulness of this approach has been the immediate objective rather than the underlying biological problems

MATERIAL AND METHODS

Material

One hundred and fifty-eight patients (89 women and 69 men) from ages 16 to 78 years were studied (Table I) All patients were selected from a general medical ward Approximately one third were chosen with the intention of studying special conditions Although the remaining patients were chosen at random some categories have been deliberately avoided These categories involved patients failing to give their consent very obese patients patients in acute and/or life threatening situations and patients with severe diabetes mellitus The majority of the patients were not seriously ill Sampling with controls was not performed in the usual manner However a reasonably good distribution in respect of sex, age and various diseases was achieved within the limits of the present material.

Sampling procedure

The sampling was constantly performed at approximately 7 30 a.m in order to avoid diurnal variations as much as possible Tissue samples were obtained from normal skin by punch biopsy in the gluteal region after local analgesia with ethylene dichloride A biopsy punch of 6 mm in diameter was used and bleeding was most unusual The weight of the tissue sample was usually in the range of 30–70 mg Weighing, wrapping in aluminium foil and storing at −25 C were carried out immediately after the tissue removal. The blood samples, using heparinized tubes were drawn from the cubital vein im

Table I *Patient material*

Group	Number of pat		
	Females	Males	Total
Adrenal or pituitary disorders	8	5	13
Treated with ACTH or glucocorticosteroids	7	8	15
Dysfunction of sexual hormones or treated with such	15	—	15
Collagen diseases incl rheumatoid arthr Not treated with glucocorticosteroids	22	7	29
Various other patients	37	49	86
Total material	89	69	158

mediately before the biopsy procedure Separation and storing of the plasma at −25 C was done within minutes after the blood was drawn The only exceptions from the above described procedure were two patients in whom ACTH tests were performed in the evening. Tissue and blood samples were obtained just before and immediately after a 4 hour i.v infusion of 70 units ACTH The second punch biopsy was done in the opposite gluteal regions

Analytical method

The analytical procedure was initiated within a few hours after the sampling simultaneously for plasma and tissue Essentially the analytical procedure (6) is a micro scale method based on a spectrophotofluorometric technique It allows for reasonable precision and accuracy in determining cortisol down to about one nanogram per sample The specificity is high and corticosterone is quantitatively removed during the extraction procedure The results are given as concentrations in ng/ml plasma and ng g wet weight tissue respectively

RESULTS

The biological validity of the method was tested in a limited number of patients in whom the results could be logically predicted Table II demonstrates a series of patients treated with ACTH or prednisone and Table III shows the results from patients known to suffer from either deficiency or overproduction of adreno-cortical steroid hormones In general the results are compatible with what could be expected low values in varying degree of adreno-cortical suppression with prednisone and in Addison s disease and high values in Cushing s disease or intensive cortisone treatment However considerable individual variations exist The results from Tables II and III seem to indicate that the tissue concentrations of cortisol determined by this method are reliable and specific to an extent to which they can be used for investigative purposes

Although no attempt has been made to measure

Table II *The concentration of cortisol in plasma and skin in patients treated with ACTH or glucocorticosteroids*

Sex	Age	Diagnosis	Medication (daily dose) and comments	Conc of cortisol	
				Plasma (ng/ml)	Tissue (ng/g)
o	57	Cancer coli metast	Meprobamate aspirin codein	(i) 33[a]	30
				(ii) 43[a]	[illegible]40
♂	38	Cardiospasm	0	(i) 53[b]	8
				(ii) [illegible]9 [b]	99
o	25	Compr vert thorac	Adreno-cortical supp test	0	0
♂	68	Panart nodosa	Prednisone 25 mg Imuran	24	35
♀	62	Autoimmune hemolyt anemia	Prednisone 20 mg	0	0
o	36	Urticaria arthralgia	Prednisone [illegible]0 mg (four days) cyproheptadine	160	70
♀	47	Sarcoidosis	Prednisone 17 5 mg	29	0
♀	41	Pulmonary fibrosis	Prednisone 17 5 mg	0	0
♀	24	Hodgkin s disease	Prednisone 15 mg, cyclophosphamide	1 1	55
o	69	Pneumonia asthma pern anemia	Prednisone 15 mg, digitalis penicillin chlordiazepoxide	0	[illegible]4
♀	38	S L.E.	Prednisone 10 mg indomethacin chloroquine	10	65
o	43	Bronchial asthma	Prednisone 10 mg theophyllamine	77	118
♀	58	Bronchial asthma bronchitis	Prednisone 7 5 mg	41	4
♀	2[illegible]	Bronchial asthma bronchitis	Prednisone (disc 12 days previously)	101	31
o	65	Bronchitis	Ledercort (disc 16 days previously)	133	101

[a] Between (i) and (ii) an i v infusion was given containing 70 units ACTH (4 h)
[b] As the patient above
[c] Four days administration of dexamethasone

Table III *The concentration of cortisol in plasma and skin in patients with present or previous disorders in the hypophysis or the adrenal cortex*

Sex	Age	Diagnosis	Medication (daily dose) and comments	Conc of cortisol Plasma (ng/ml)	Conc of cortisol Tissue (ng/g)
♀	55	Addison s dis	Cortisone 50 mg fludrocortisone 0 2 mg No cortisone for 3½ days	0	0
♀	71	Addison s dis	No treatment	5	0
♀	43	Addison s dis	Cortisone 25 mg fludrocortisone 0 1 mg No cortisone for 24 hours	0	190
♂	19	? Addison s dis diabetes	Insulin	53	93
♀	64	Hypophysectomized	Cortisone 150 mg thyr horm penicill Adrenal crisis two weeks ago	227	179
♂	37	Cushing s dis	Hydralazine thiazide	380	76
♀	41	Previously treated Cushing s dis	(i) Cortisone 12 5 mg	(i) 110	83
			(ii) No cortisone for 12 days	(ii) 136	36
♀	64	Previous Cushing s dis	Guanethidine thiazide Clinically normal	160	23
♂	73	Previous Cushing s dis	Papaverin Clinically normal	155	58
♂	38	Previous Cushing s dis	0 Clinically normal	137	65
♀	45	Previous Cushing s dis	Trixal thiazide Clinically normal	146	61
♂	37	Adrenal steroid metabolism defect	0 Huge excretion of 17 keto-steroids Clinically normal	130	56
♂	15	Hypophys gigantismus	X ray on the hypophysis	130	52

the different plasma fractions of cortisol a positive correlation between the corresponding values for total concentrations in plasma and in tissue should be readily expected A highly selected group of patients was chosen to avoid the influence of irrelevant" factors (see later) Thus the corresponding values shown in Fig 1 derive from 48 male patients not suffering from endocrine or collagen diseases nor treated with hormones In spite of considerable individual variation a medium grade of positive correlation can be demonstrated ($r = 0\,443$) In contrast an attempt to correlate the tissue/plasma ratio to the age in the same group gave a negative result (Fig 2)

The average values for 81 patients (48 men and 33 women) are given in Table IV The group includes all ages and a variety of diseases and treatments but excludes patients with diseases in the adrenal cortex, the hypophysis the connective tissue or the reproductive organs as well as patients undergoing hormonal therapy The average results for plasma and tissue cortisol are slightly but non significantly higher for the females

The possible influence of oestrogen has been illustrated in Table V Treatment with oral contraceptives (and thus oestrogens) raised the plasma concentration about 2½ fold while the tissue concentrations remained unchanged Thirty three

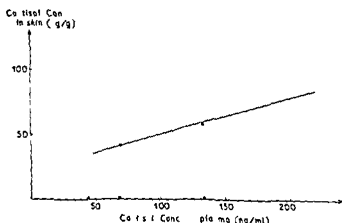


Fig 1 The concentration of cortisol in skin (ng/g) is plotted against the plasma cortisol concentration (ng/ml) for 48 male patients not suffering from endocrine disorders or collagen diseases nor treated with hormones A medium grade of positive correlation was found (regression coefficient = 0 443) The regression line is $y = 1\,43 + 0.285\,x$

Table IV *The concentrations of cortisol in plasma and skin in patients without collagen diseases dys function in the sexual or adreno-cortical hormone systems nor undergoing hormonal treatment*

Sex	Age range	No of pat	Plasma (ng ml) ± S D	Tissue (ng/g) ± S D
♂	16–78	48	117 1 ± 43 1	54 9 ± 27 3
♀	16–69	33	130 1 ± 4 7	60 2 ± 41 3
♂ + ♀		81	122 4 ± 43 4	57 0 ± 33 8

Table V *Relation of oestrogenic influence to the concentration of cortisol in plasma and skin in women*

Group	No of pat	Plasma (ng/ml) ± S D	Tissue (ng/g) ± S D
Treated with oral contracept. Age 20–41 years	8	308 9 ± 96 9[a]	65 5 ± 26 7
Thirty three female patients from Table IV			
I 16–40 years No hormones	9	133 4 ± 49 7[a]	75 6 ± 46 2[b]
II >40 years either still menstr or menopause < 2 years	11	127 8 ± 31 2	50 9 ± 49 2[b]
III Menopause > 2 years	13	129 1 ± 49 1	58 2 ± 30 2[b]

[a] The difference is statistically significant at $p < 0.01$

[b] The difference is not significant (Duncan s multiple range test) Compare Fig 3

Table VI *The concentration of cortisol in plasma and skin in five women with dysfunction in the sexual hormone secretion or in treatment with sex hormones*

Age	Diagnosis and comments	Conc. of cortisol Plasma (ng/ml)	Tissue (ng/g)
24	? S L.E. Receives oral contraceptives (not been taken for 6 days)	89	83
59	Irritable colon and climacterium Oestrogen inj. every 4th week. Last inj 25 days previously	190	7.
24	Pregnant in the 7th month	155	30
24	Anorexia nervosa Menostasia for 2 years. Chlorpromazine 150 mg daily	116	0
28	Stein Leventhal's syndrome Migraine medication dihydroergotamine	101	37

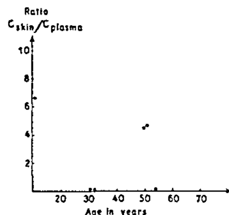


Fig . For the same 48 male patients as in Fig. 1 the ratio between the cortisol concentration in skin and in plasma is plotted against age No correlation could be demonstrated

women were divided into three arbitrary groups supposedly reflecting different levels of endogenous oestrogen production No difference in the plasma values was noted among the groups but a tendency to lower concentrations of tissue cortisol in the years around the menopausal age seems clear Because of the large individual variations the difference is not statistically significant in this small number of patients (Duncan s test at the 5 % level) Fig 3 shows the individual plottings of tissue/plasma ratios for the three groups As can be seen 8 out of 11 patients in the "menopausal" group showed exceptionally low values. In Table VI an attempt to illustrate the influence of oestrogen on the distribution of cortisol has

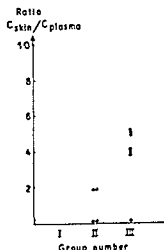


Fig 3 For 33 females the ratio cortisol in skin (ng g)/ cortisol in plasma (ng/ml) is shown for group I < 40 years group II > 40 years either still menstruating or menopause for < 2 years and group III menopause > 2 years.

Table VII *The concentrations of cortisol in plasma and skin in various groups of patients*

Groups	No of pat	Plasma (ng/ml) ± S D	Tissue (ng/g) ± S D
Various diseases and drugs totally (from Table IV)	81	122 4 ± 43 4	57 0 ± 33 8[a]
Special groups			
Hepatic cirrhosis	6	146 8 ± 60 5	108 5 ± 46 0[a]
G I dis.	12	131 4 ± 49 6	47 0 ± 48 0
Cardiovascular dis	12	112 0 ± 48 0	55 0 ± 30 7
Renal and urinary tract dis	10	128 1 ± 47 8	65 9 ± 32 5
Hematological dis	7	110 3 ± 48 1	41 0 ± 34 3
Pulmonary dis	9	106 3 ± 36 5	48 1 ± 33 4
Non rheumatic joint dis	8	130 3 ± 5 [illegible]	49 0 ± 20 6
Chlordiazepoxide or diazepam	11	134 3 ± 37 2	48 6 ± 37 8
Various anti psychotic drugs	12	123 4 ± 28 3	64 9 ± 30 5
Collagen diseases including R.A	21	136 2 ± 55 7	78 2 ± 44 0[a]
Indomethacin 75–125 mg daily	9	92 2 ± 31 1	36 6 ± 34 1[a]

[a] The difference is statistically significant $p < 0\ 05$

been made by presenting five female patients in various hormonal conditions These patients are listed separately as they are not included in other groups

In Table VII all 31 patients from Table IV are grouped according to the organ or system in which their actual disease is diagnosed No concern is paid to clinical entities age sex or therapy Only special groups large enough to be representative are listed but some overlapping exists Two groups of *drug treatments* are added as many patients did not in fact receive drugs at the time of the biopsy No significant correlation seems to exist between this crude classification and the observed cortisol concentrations The only exception is a small group of patients with hepatic cirrhosis whose tissue concentration of cortisol was significantly higher than that of the rest of the group A separate *group* of 21 patients with various collagen diseases (including rheumatoid arthritis) had on the average higher tissue levels than patients with out collagen diseases but the difference is not significant The comparability of the two groups may be questioned but the group with collagen diseases as would be expected was not biased by

Table VIII *Patients having a higher concentration of cortisol in the skin than in the plasma (see text for details)*

Sex	Age	Diagnosis	Medication and comments	Conc of cortisol: Plasma (ng/ml)	Conc of cortisol: Tissue (ng/g)
♀	69	Bronchial asthma pneumonia pern anemia	Prednisone 15 mg, digitalis penicillin chlordiazepoxide	0	24
♀	43	Addison s disease	Cortisone 25 mg fludrocortisone 0 1 mg No cortisone for 24 h	0	190
♀	38	S L E	Prednisone 10 mg indomethacin chloroquine 250 mg	10	65
♂	68	Panarteritis nod	*Prednison 25 mg Imuran*	[illegible]4	35
♀	58	Bronchial asthma	Prednisone 7 5 mg	41	42
♀	40	Prim amenorrhea	0	49	9[illegible]
♀	19	? Addison s disease diabetes	Insulin	53	93
♀	43	Bronchial asthma	*Prednisone 10 mg* theophyllamine	77	108
♀	51	Mb cord nervosism	Chlordiazepoxide stemetil digoxine	89	171
♀	56	Bronchial asthma	0	91	101
♀	21	Observatio obese	0	97	115
♀	56	Hepatic cirrhosis	0	57	146
♀	19	Pubertas retarda	Diazepam	89	285
♀	37	Rheumatoid arthr	*Truxal chloroquine 50 mg*	150	1038
♀	17	Urticaria	0	150	273
♀	63	Paraproteinemia	0	155	790
	73	S L E	Chloroquine 250 mg	173	204

Table IX *The concentration of cortisol in plasma and skin in five patients receiving treatment with chloroquine*

Sex	Age	Diagnosis	Medication	Plasma (ng/ml)	Tissue (ng/g)
♀	34	S L E	Chloroquine 250 mg Prednisone 10 mg Indomethacin 50 mg	10	65
♀	32	Rheum arthr	Chloroquine 250 mg Truxal 45 mg	150	1038
♀	73	S L E	Chloroquine 250 mg	173	204
♀	40	Hemolyt anemia Hypothyroidism	Chloroquine 250 mg Thyroidin 1.0 mg	165	0
♀	41	S L E	Chloroquine 250 mg	101	65

an over representation of young women which would have influenced the average cortisol level The treatment with indomethacin is accompanied by a statistically significant fall in the tissue cortisol when compared to the group of untreated cases of connective tissue diseases However the correctness of such a comparison may be disputed from both a statistical and a biological stand point

A set of observations not fully understood is listed in Table VIII In 17 out of 158 patients a higher concentration was found in tissue (per gram wet weight) than in plasma (per ml) These patients are not incorporated in other groups In a majority of the 17 cases with reversed ratio the differences between plasma and tissue values are not very large but in a few cases considerable Analytical errors are not likely but unfortunately re biopsies were not performed Three out of the 17 patients were being treated with chloroquine and therefore five patients receiving this drug (of the total 158 patients) are listed individually in Table IX Investigations in our laboratory have confirmed that chloroquine as such does not interfere with the determination of cortisol in the used analytical procedure However it is not known whether this is the case for any of the four or five metabolites chloroquine is known to produce

DISCUSSION

Application of the method

Determinations of cortisol other than in the adrenal cortex and plasma are few in number and radio isotopic methods have been preferred (2 4 8 12) Actual tissue concentrations of cortisol in humans performed by chemical determinations do not seem to have been studied previously Consequently the results from the present investigation cannot be compared directly with others but from the results presented in Tables II and III it seems reasonably acceptable that the method is capable of reflecting the actual content of cortisol in the tissue sample The applicability of the method for correct determination of plasma levels is obvious It is however a matter of dispute whether the figures for the tissue given as a concentration of cortisol in the total tissue is a biologically correct expression and whether or not this can be compared to the plasma concentration Reasons for this possible limitation are that the distribution space for cortisol is in fact not defined for the tissue (skin and subcutaneous tissue) used in this investigation Furthermore as pointed out by Plager et al (1) the form in which cortisol exerts its physiological activities in the tissues is not known and it should be added that the concentration—at tissue level—necessary for its normal activity is also unknown Finally the present method like many others gives no information concerning the rate of turnover of cortisol in the tissue for which reason conclusions based upon tissue concentrations alone could be grossly misleading with respect to the dynamics of this hormone

The influence of oestrogen

Previous studies of the influence of drugs and diseases on the distribution pattern of cortisol in the body have predominantly been approached from the point of view of plasma protein binding The influence of oestrogen has attracted considerable interest after the observation was made that oestrogens sharply increased the plasma cortisol concentration (2 4 11 13 20 21) It is a remarkable characteristic that clinical evidence of hyper corticism is not present in these patients

(9 10) These patients are found to have an increased transcortin bound fraction of plasma cortisol as well as transcortin itself However according to several investigators the free cortisol remains unchanged (14 15 17 18 20) It is therefore usually assumed that the tissue concentration of cortisol also remains unchanged as only free plasma cortisol is believed to be in equilibrium with tissue cortisol However the oestrogen-cortisol relationships may be more complex than implied in the above cited works as stressed by Plager et al (11) who also found the free fraction of plasma cortisol increased during oestrogen treatment while the rate of cortisol production was unchanged Furthermore Sandberg et al (15) have stated that about 50% of the total amount of transcortin is located outside the vascular bed To what extent cortisol is bound to this portion of transcortin remains to be clarified but only non protein bound cortisol is physiologically active in the tissue (1 7) Bradley and Waterhouse (2) confirmed in patients that the total extravascular pool of cortisol was unaffected by oestrogen while the intravascular concentration was increased significantly As ACTH stimulation increased both they concluded that the kinetics of cortisol was changed by oestrogen consistent with an increase in the transcortin level The actual extravascular concentration could not be calculated because the distribution space was unknown

The results from the present investigation confirm that the total plasma concentration of cortisol is increased during the treatment with oral contraceptives (and thus oestrogens) but that the concentration of tissue cortisol is unaffected Hence direct tissue determinations are in accordance with the previous observations which show that conditions with elevated oestrogen affect the distribution pattern of cortisol in the body As no distinction was made between bound and free plasma cortisol the relative shift in these fractions was not recorded in our study However the results seem to indicate that the net movement of cortisol from plasma to tissue is unaltered providing an unchanged turnover of cortisol in the tissue

In order to study the possible influence of physiological variations of oestrogen simultaneous oestrogen and cortisol determinations should ideally be performed Such results are not available The material in the present investigation was too small for an attempt to relate the concentrations of plasma and tissue cortisol in younger women to the time in the menstrual cycle Another approach was made by dividing 33 women into three arbitrary groups hopefully reflecting different levels of oestrogen The younger females presumed to have a higher average oestrogen production also had the highest concentration of cortisol in the tissue whereas the women centered around the menopausal age showed the lowest average concentration The plasma levels were similar in the three groups but Fig 3 clearly demonstrates that the majority of low tissue/plasma ratios are found in the years around menopause A similar variation was not seen in males (Fig 2) These observations although grouped slightly differently were published in a preliminary report (16) The results strongly suggest that the distribution of cortisol in females is also influenced by the endogenous oestrogen production This observation is however difficult to explain only on the basis of a shift in the free and bound portions of plasma cortisol If this were the case a relatively larger part of the total plasma cortisol should be bound to protein at a lower oestrogen level which is unlikely The decrease in tissue cortisol in women around the menopausal age is not associated with any clinical manifestation of hypocorticism indicating no change in the physiological active cortisol It might therefore be suggested that cortisol exists in the tissue in at least two different forms one of which is inactive and presumably bound to a protein Speculatively a temporary decrease in such a cortisol binding protein in the tissue could be caused by the lack of oestrogenic stimulation on the protein production resulting in a decrease in the total tissue concentration of cortisol with no change in the cortisol activity

Connective tissue disorders

The rheumatic diseases may possibly alter the metabolism and distribution of corticosteroids in the connective tissue although most investigators have failed to clearly demonstrate such a relationship (5 22) The present investigation suggests a tendency to higher tissue concentration of cortisol in patients with collagen diseases and rheumatoid arthritis This tendency however is not significant but the decrease in tissue cortisol observed after the treatment with indomethacin in such cases is striking Indomethacin was the only

non steroid anti inflammatory agent studied and although the findings seem to be fairly consistent much more work needs to be done to confirm this observation Brodie (3) suggested on the basis of corticosterone determinations in rats that non steroid and anti inflammatory drugs exceeded their activities by an increase of the unbound plasma steroid and therefore indirectly raised the tissue concentration of the active hormone Our results do not support this hypothesis However as nothing is known about indomethacin s influence on the metabolism of cortisol in the tissues an indirect action of this agent via steroids cannot be completely ruled out The results presented in this study do suggest a relationship between the action of indomethacin and the distribution of cortisol Alterations in the properties of tissue binding of cortisol might also be suggested

Other conditions

A relatively impaired steroid metabolism in the liver may be a reasonable explanation of the higher concentration of cortisol found in the skin of patients with cirrhotic livers but the number of patients is too small for any definite conclusion More attention should be directed to the finding that approximately one tenth of the total material showed a higher concentration of cortisol in the tissue than in the plasma About two thirds of these cases may be seen in the light of their special hormonal conditions or some may even be explained on the basis of experimental variations as the difference between the tissue and plasma values is not very large A group of six patients in which the reverse difference is considerable is not easily explained It confirms the impression however that cortisol (evidently in an inactive form) can exist in a high concentration in the tissue with no relation to the simultaneous lower plasma concentration The observation that three such reverse ratios were found among totally five patients treated with chloroquine may suggest a connection between this agent and a peculiar tissue binding of cortisol

The discrepancy between corresponding plasma and tissue concentrations is a conspicuous finding in the present material A moderate degree of positive correlation can only be demonstrated when many factors believed to influence the distribution of cortisol are excluded (Fig 1) In fact the observations from the entire material and the special examples discussed above leave the impression that the two sets of simultaneous determinations cannot really be related to each other Relative changes in the free and protein bound plasma cortisol seem only to a certain degree to explain the discrepancy so that additional factors must be present Only about 10 % of the plasma cortisol is free i e diffusable (20) On the average however tissue cortisol is found to be about 50 % of the plasma concentration The difference between the 10 % diffusable cortisol and the 50 % actually observed in the tissue cannot be explained only on a basis of different distribution spaces This also points to the possibility of more factors involved in the distribution of cortisol than the plasma protein binding Some of the results suggest that the variations in the tissue concentration are time related a phenomenon well known for plasma but the tissue variation might be out of phase with the fluctuations in the plasma concentration If so cortisol can be retained in the tissue in spite of a decreasing plasma concentration The observations made in the present investigation taken in conjunction with the results of Plager et al (11) therefore suggest that plasma and tissue concentrations of cortisol may vary partly independently of each other Undoubtedly the tissue cortisol is supplied by the circulating pool of the hormone and the active concentration of tissue cortisol can be increased rapidly from the free plasma cortisol But other slower acting mechanisms also seem to influence the distribution pattern Such mechanisms may be located in the tissue and they seem to influence the distribution of cortisol within the tissue as well as across the vascular membrane without necessarily or primarily affecting the biological activities of the hormone

ACKNOWLEDGEMENTS

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REFERENCES

1 Blecher M Endocrinology 79 541 1966
2 Bradley E M & Waterhouse C J clin Endocr 26 705 1966
3 Brodie B B Proc roy Soc Med 58 946 1965
4 Daughaday W H Adler R E Mariz, I K & Rasinski D C J clin Endocr 2_ 704 196?
5 Gray C H & Shaw D A J Endocr 33 33 1965

6 Jansen J Aa Hvidberg, E & Schou, J Scand J clin Lab Invest 20 49 1967
7 Matsui, N & Plager J E Endocrinology 78 541 1966
8 Migeon C J., Sandberg A A Decker H A Smith D F Paul A C & Samuels L. T J clin Endocr 16 1137 1956
9 Mills L H Schedl H P., Chen P S., Jr & Bartter F C J clin Endocr 20 515 1960
10 Peterson, R. E Recent Progr Hormone Res 15 231 1959
11 Plager J E Schmidt K. G & Staubitz, W J J clin Invest. 43 1066 1964
12 Rauschkolb R. R. & Rauschkolb E W J invest Derm 37 1 1961
13 Robertson M E. Stiefel M & Laidlaw J C J clin Endocr 19 1381 1959
14 Sandberg A A & Slaunwhite W R., Jr J clin Invest 38 1290 1959
15 Sandberg A. A Woodruff M Rosenthal H., Nienhouse N & Slaunwhite W R. Jr J clin Invest 43 461 1964
16 Schou J., Jansen J Aa & Hvidberg E Nature 215 0 1967
17 Seal U S & Doe R. P J biol Chem 237 3136 1962
18 Slaunwhite W R., Jr & Sandberg, A A J clin Invest. 38 384 1959
19 Tait J F & Burstein S In vivo studies of steroid dynamics in man. In The hormones (ed G Pincus K. V Thimann and E B Astwood) Academic Press, New York 1964
20 Taliaferro I Cobey F & Leone L Proc Soc exp Biol 92 742 1956
21 Wallace E Z., Silverberg, H I & Carter A C Proc Soc exp Biol 95 805 1957
22. Winter J A., Sandberg, A A & Slaunwhite W R., Jr Arthr and Rheum 9 389 1966

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SYSTEMIC ARTERIAL HYPERTENSION

Aspects of Etiology and Pathogenesis in a Retrospective Study of a Hospital Material

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Abstract Among 301 cases of unselected hypertension 45 proved to be of the essential and the remaining 55% of the secondary type A sex difference was present in that the essential type was found in 53% among men against 33 among women The major causes of secondary hypertension were chronic pyelonephritis and nephritis Cushing s disease and allied disorders and aortic coarctation In a series of 177 patients with selected hypertension 76 maintained the diagnosis of essential hypertension No significant sex difference was observed All the cases of secondary hypertension were of renal origin chronic pyelonephritis being responsible for more than 50% of the cases Of the techniques employed urography with early sequential exposures seemed to be the method of choice for screening purposes Renal biopsy was similarly helpful in obtaining the correct diagnosis Renal angiography also proved to be of great significance and its use should clearly be extended The presently available methods for evaluating systemic arterial hypertension are discussed

Recent advances in methods of study have served to clarify the etiology of systemic arterial hypertension Secondary hypertension in which the cause of the blood pressure elevation can be demonstrated has increased while the so-called essential hypertension has proportionately declined

Although this is widely recognized the extent of this change is little known

The present study provides information on the etiology of arterial hypertension as obtained in a department with access to most modern methods of investigation

The study has been conducted along two lines Firstly an analysis has been made of the total number of patients who during the observation period had sustained hypertension regardless of their main disease Secondly a similar analysis has been carried out of those patients who were exclusively admitted for evaluation of systemic arterial hypertension The study thus comprises unselected as well as selected cases of hypertension

MATERIAL

Included in the study were all patients with sustained hypertension and with blood pressure above 150 100 mm Hg and who were admitted during 1965 1966 and the first five months of 1967

Ages ranged from 15 to 69 years with an identical mean age of 51 years for both sexes

A great number of the patients were referred from other hospital for further evaluation in a University clinic So far the material is to be regarded as selected

METHODS

In addition to routine clinical examinations the patients were investigated by means of urography with early sequential exposures (9) radioisotope renography and when necessary renal angiography and renal biopsy Differential renal function tests were not consistently carried out Angiotensin infusion tests were not at all employed due to their doubtful significance (?) The department had no access to methods for estimation of the pressor substances renin and angiotensin Primary aldosteronism was searched for by ordinary electrolyte studies and by assessing urinary output of aldosterone In the majority of the cases catecholamines and steroids were estimated in the urine

Essential hypertension was classified according to the system suggested by Rasmussen (14) Following this system the malignant type is characterized by the presence of papilledema while the levis type shows only minor retinal vascular changes and no damage of the target organs In between these two types the gravis type is situated

RESULTS

It can be observed that less than half of all the patients suffered from the essential type of hyper

Table I *The series of unselected arterial hypertension and its distribution in subgroups*

		Essential		Secondary	
		No		No	
Men	172	91	53	81	47
Women	129	43	33	86	67
Total	301	134	45	167	55

Table II *The underlying diseases of secondary hypertension*

	Men	Women
Chronic nephritis	38	17
Chronic pyelonephritis	8	18
Cushing s disease	7	14
Aortic coarctation	7	11
Renal artery stenosis	7	2
Pheochromocytoma	1	1
Hyperparathyreoidism	0	2
Varia	13	21
Total	81	86

Table III *The series of selected arterial hypertension and its distribution in subgroups*

		Essential		Renal	
		No		No	
Men	115	91	80	24	20
Women	62	43	70	19	30
Total	177	134	76	43	24

tension (Table I) Among women only one third had this type

As expected chronic nephritis and pyelonephritis were responsible for a major part of the secondary types of hypertension (Table II) The sex linked preponderance of these disorders is clearly illustrated Reservations have to be made for the many cases of Cushing s disease Only one third were new cases another third were cases admitted for control whereas the remainder consisted of cases in which the diagnosis was under suspicion Aortic coarctation is also over represented in the series a mere consequence of the fact that the department is mainly occupied with cardiovascular disease *Pheochromocytoma* was only found in less than 1% Being a curable form

Table IV *The underlying diseases in renal hypertension*

		Chronic pyelo nephr	Chronic nephritis	Renal artery stenosis
Men	25	8	10	7
Women	18	14	2	2
Total	43	22	12	9

of hypertension however its detection remains essential A considerable amount of the secondary hypertension (*varia*) comprised collagen disorders coronary and valvular heart disease peripheral vascular disorders and diabetes mellitus There were three cases of polyarteritis nodosa Not a single case was encountered of primary aldosteronism or bilateral cystic disease of the kidneys

A total of 177 patients were *admitted exclusively* for evaluation of systemic arterial hypertension (Table III) The vast majority were men A careful study of all these patients gave as a result that 76% maintained the diagnosis of essential hypertension the remaining 24% being hypertension of renal origin

A majority of 64% of the essential hypertensions were of the gravis type whereas only 6% fulfilled the criteria for malignant hypertension (14)

In renal hypertension chronic pyelonephritis most often was the underlying disease accounting for 51% of the cases (Table IV) Chronic nephritis and renal artery stenosis were responsible for 29 and 10% respectively For the two first mentioned disorders the typical sex linked preponderance is clearly shown If related to the whole series of 177 patients the three renal disorders above were responsible for 12 7 and 5% of the cases

Table V *The total number and the results of investigations undertaken in 170 patients with selected arterial hypertension*

	Total		Pathol		Failures	
Urography	115	68	47	40	16	14
Renograhy	123	72	77	63	31	25
Renal angiography	68	40	42	62		
Renal biopsy	23	14	20	87		

of arterial hypertension An extended use of renal angiography would certainly have produced an increase of hypertension due to renal artery stenosis

Seven patients were adequately examined prior to the admission The number and the results of the various investigations undertaken in the remaining 170 patients are presented (Table V) Urography was only conducted in two thirds of the patient group This was due to omission of this examination in nearly all the patients having the levis type of hypertension As both chronic pyelonephritis and renal artery stenosis can exist with normal urinary findings and with blood pressure falling to normal values during hospitalization it is mandatory that urography be carried out in all cases of hypertension

Renal angiography was undertaken in only 40 % of the patients Probably a more frequent use of this method would have brought forward a greater number of renal hypertensions

Urography as well as renography provided several erroneous results urography by missing positive findings renography by detecting far too many cases with apparently significant reduction of renal function and renal blood flow

Disparity of the length and size of the kidneys was a consistent finding in chronic pyelonephritis whereas such disparity was present in only four out of nine cases of renal artery stenosis (Table VI) Aplasia of one kidney was found in five cases by means of urography Subsequent renal angiography proved two cases of aplasia to be due to complete stenosis of the ipsilateral renal artery (Table VII) The condition known as fibromuscular hyperplasia was found to be present in the renal artery of a woman Systolic bruits over the actual renal artery were heard in less than half the cases of renal artery stenosis

Of 23 renal biopsies three were unsuccessful Of the remaining biopsies 17 were undertaken for diagnostic purposes only (Table VIII) The questioned diagnoses naturally were chronic nephritis and pyelonephritis sarcoidosis and amyloidosis In ten instances biopsy was undertaken of the diseased or most affected kidney and in seven instances of the presumably healthy one The latter were carried out to determine operability For the same reason biopsy of the contralateral kidney was made in three instances when unilateral renal artery stenosis was present

Table VI *Findings in 47 urographies with pathological result*

Small kidney on one side	10
Small kidney on both sides	3
Renal cyst	3
Stone	2
Tumor	5
Aplasia of one kidney	5
Delayed "appearance time" on one side	5
Delayed "appearance time" on both sides	5
Anomalies of calyces pelvis ureter	9

Table VII *Findings in 42 renal angiographies with pathological result*

Renal artery stenosis on one side	6
Renal artery stenosis on both sides	6
Renal artery occlusion on one side	
Fibromuscular hyperplasia of one renal artery	1
Aplasia of one kidney	3
Renal artery aneurysm on one side	[illegible]
Atherosclerosis of abdominal aorta	1
Renal cyst	4
Small kidney on one side	10
Small kidney on both sides	5

Table VIII *Findings in 20 renal biopsies with pathological result*

Chronic nephritis	2
Chronic pyelonephritis	7
Renal arteriolosclerosis	10
Renal arteriosclerosis	1

The biopsy specimens most often provided the correct diagnosis However a great number only showed arteriolosclerosis compatible with hypertension but without specified diagnosis A majority of these specimens belonged to kidneys with otherwise clear signs of chronic pyelonephritis Knowledge of clinical data therefore seems necessary for correct interpretation

In the three biopsies from the contralateral kidney in patients with renal artery stenosis on one side significant arteriolar damage was found Consequently no surgery was undertaken In two patients biopsy was done on both kidneys first for diagnosis thereafter to determine operability No serious complications followed the performance of biopsy of the kidneys in the present series

A study was also made of age duration of the hypertension its acceleration renal function and electrolytes No significant differences in these respects were found between the various types of hypertension Naturally the target organ damage

corresponded with the severity of the disease Hypokalemia similarly occurred more often in severe hypertension both spontaneously and as a result of treatment with diuretics

Although regarded as unspecific the symptoms produced by hypertension are unquestionable More than two thirds of the patients with essential hypertension had subjective complaints Usually these symptoms were relieved by adequate lowering of the blood pressure Symptomatology was of the same order and nearly of the same severity in both severe and mild hypertension but notable breathlessness and tiredness always signaled serious hypertension

COMMENTS

Recent studies have shown that systemic arterial hypertension even in mild form bears a gloomy long term prognosis and that early treatment reduces mortality to a considerable extent (5 15) The success of medical treatment is closely related to the height of the blood pressure at the outset of treatment (13) Furthermore the response to such treatment is equally good regardless of the cause of the hypertension However important these items of information may be a proper evaluation of the patient with hypertensive disease still remains fundamental for arriving at the correct diagnosis and treatment Current studies of etiology are necessary for disclosing basic knowledge of systemic arterial hypertension

Over the past years such studies have mainly concentrated upon the frequency of renovascular hypertension providing figures from 1 to 20 % The extremes in this respect appeared recently in two simultaneous communications In one of them 111 of 180 cases of hypertension were found to be caused by various types of renal arterial stenosis (10) The other failed to demonstrate any case of such a stenosis among 50 consecutive patients with unselected hypertension (1) despite of the fact that in 15 patients the disparity of the differential renal function test was typical of the presence of significant renal arterial obstruction (16)

In the present series of unselected hypertension the cause of the disease could be demonstrated in more than half of the patients A significant difference between the sexes was apparent Among men every second case was of the essential type while this was so in only one third of the women The reason seemed to be a preponderance in women of chronic pyelonephritis Cushing s disease and allied disorders collagen diseases and diabetes

Quite another picture of the etiology was brought forward by a study of selected hypertension Following a careful evaluation a total of 76 of the patients still maintained the diagnosis of essential hypertension and no significant sex difference could be observed Moreover all the remaining cases (24 %) proved to be hypertension of renal origin

Separation of selected from unselected hypertension as made in this study thus provides results which a e at great variance and thereby stress the heavy influence of selection For comparative purposes such a procedure is an advantage At present comparison between published series is more or less impossible due to ill defined terms of selection

The selected hypertension results of the present series compare favorably with two recent Scandinavian series (6 17) In all three studies the cause of the hypertension could demonstrated in nearly 30% of the cases the remaining 70% being cases of essential hypertension However among the latter are many cases which cannot be regarded as adequately examined The proportion fulfilling the more strict denomination pure essential hypertension consequently becomes much less There is little doubt that the group of essential hypertension could be considerably reduced by complete examination of all the patients regardless of the severity of the disease Renal angiography was for instance undertaken only in 45 % of the patients of the Swedish series (6) in 33 % of the series from Molde Hospital (17) and in 40 % of the present series

In other respects comparison between the above series is hardly possible due to various factors of selection However it is worth while to consider the over representation of chronic pyelonephritis and aortic coarctation and the under representation of renal artery stenosis in the present series of unselected hypertension compared to the Swedish series (6)

The validity of methods is of prime importance for studies of etiology In the present work urography with early sequential exposures was extremely helpful Radioisotope renography added little information beyond that obtained by means

of urography In fact renography led to a number of misleading observations This is no wonder as nobody has yet proved what renography really reveals in respect of renal function (8)

Differential renal function tests were not consistently employed in this study because the reliability of these tests in predicting the presence of renal artery stenosis (16) cannot be trusted (1) In addition the ureteral catheterization represents a source of infection Nor can the differential tests decide the causal relationship between the arterial stenosis and the coexisting hypertension (7 10)

This is also true of renal angiography However this method has the advantage of always providing reliable information on the patency of the renal arteries Despite the fact that significant artery obstructions have been found in normotensive individuals (4) demonstration of such lesions remains a keystone in the evaluation of hypertension

Renal biopsy is extremely helpful in arriving at the correct diagnosis (7) In reflecting the severity of the hypertensive disease and in predicting the result of surgery upon blood pressure however the value of renal biopsy must be seriously questioned (10 12)

Estimation of renin and angiotensin has hitherto been regarded as the only means by which the causal relationship between renal ischemia and hypertension could be established However studies of human renal and essential hypertension have yielded conflicting results so far (11) Similarly the angiotensin infusion test has proved more or less useless in separating essential from renal hypertension (2)

Further progress in evaluation of hypertension clearly requires better methods than those available at present This also applies to the diagnosis of primary aldosteronism which was not found in this series a fact incompatible with the frequency of this condition according to Conn (3)

REFERENCES

1 Baldwin D S van den Broek H Harnes J R, Anelas R D McManus, J E & Cassel E J Renovascular hypertension in unselected patients Arch intern med 1 0 176 1967

Bergne R Voudoukis I J & Smith, R F Angiotensin infusion tests and renovascular hypertension Clin Res 15 405 1967

3 Conn J W Normokalemic primary aldosteronism J Amer med Ass 195 1 1966

4 Eyler W R Clark M D Garman J E Rain R L & Meininger D E Angiography of renal areas including comparative study of renal arterial stenosis in patients with and without hypertension Radiology 78 879 1962

5 Gifford R W Jr Hypertensive vascular disease Effect of antipressor therapy on the course and prognosis Amer J Cardiol 17 656 1966

6 Hood B & Bjork, S The diagnosis essential hypertension Acta med scand 181 63 1967

7 Kincaid Smith P The diagnostic value of renal biopsy in renovascular and other forms of renal hypertension Antihypertensive therapy Springer Berlin 1966

8 Klapproth H J Hirakawa A & Corcoran A C Functional significance of the radioisotope renogram Experimental study J Urol 87 77 1962

9 Maxwell M H Gonic H C Wiita, R & Kaufman J J Use of the rapid sequence intravenous pyelogram in the diagnosis of renovascular hypertension New Engl J Med 270 213 1964

10 Maxwell M H Lupu A N & Franklin S S Clinical and physiological factors determining diagnosis and choice of treatment of renovascular hypertension Circulat Res Suppl 2 01 1967

11 McPaul J J Jr McIntosh D A Williams L F Gritti E J & Grollman A Correlation of the pressor activity of the renal venous effluent with excretory function and other tests in focal parenchymal and vascular renal disease Circulation 33 781 1966

12 Mendonca P P de & Young J D Jr Renovascular status after renal surgery for hypertension J Amer med Ass 201 592 1967

13 Moyer J H & Brest A N The changing outlook for the patient with hypertension Amer J Cardiol 17 673 1966

14 Rasmussen, H Inndeling a diastolisk hypertensjon Nord Med 46 1847 1951

15 Smirk, H F Prognosis in retinal grad 1 and 2 patients Antihypertensive therapy Springer Berlin 1966

16 Stamey T A Diagnosis of curable unilateral renal hypertension by ureteral catheterization Postgrad Med 9 496 1961

17 Yttrehus K. Undersøkelse av hypertensjonspasienter i Molde Nord Med 1 9 1 9 (Abst) 1968

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IDIOPATHIC RETROPERITONEAL FIBROSIS

A Case of an Unusual Localization Effectively Treated with Glucocorticoid

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Abstract A case of idiopathic retroperitoneal fibrosis of an unusual site at the upper pole of the right kidney is reported The case was confirmed histologically and treated with glucocorticoid for nine months This resulted in definite clinical improvement and complete normalization of the elevated ESR and abnormal serum electrophoresis Compression of the inferior vena cava demonstrated by cavography was significantly less marked after the corticoid medication The patient has been followed for eight months after discontinuation of the treatment and throughout this period he has remained symptom free and fully capable of working. As a conclusion from this case of idiopathic retroperitoneal fibrosis and from reports in the literature the author feels that all cases of idiopathic retroperitoneal fibrosis should be treated with glucocorticoid in high doses over a long period also when surgery is indicated

Idiopathic retroperitoneal fibrosis (IRF) is a rare condition It has been known since 1948 (14) but its aetiology is still partially unknown Recently it has been related to the intake of methysergide bimaleate (Deseril®) (3)

The disease most often affects males between 40 and 60 years of age The clinical picture is uncharacteristic consisting of fatigue weight loss gastrointestinal symptoms and low back pain There may be fever anaemia and an elevated ESR

These symptoms and laboratory findings correspond to the active period of the disease when the morbid process is spreading in the retroperitoneal space This is followed by a phase of healing which may give rise to ureteral compression and consequently signs of obstructive uropathy Pyelography at this stage shows a characteristic appearance of medially displaced and narrowed ureters

Pathologically the disease presents itself as a 2–5 cm thick fibrous infiltration localized on the posterior abdominal wall on a level with the bifurcation of the aorta The lesion extends laterally to the ureters which it envelops

Proximally it seldom spreads farther than the lower poles of the kidneys and the distal limit is usually the promontory of the sacrum

Histologically the appearances are predominated by firm collagen connective tissue with lymphocytes and plasma cells In addition there may be polymorphonuclear leukocytes and fatty vacuoles Thus the histological picture is non specific

Surgical treatment usually consists in freeing and peritonealization of the ureters Nephrostomy or nephrectomy may be necessary in the more advanced stages Previously reports have been published of X ray irradiation (8 12) antiphlogistics (6 9) cytostatics (8) and antibiotics but without any convincing effect

Treatment with glucocorticoid has also been reported by a few authors (1 2 10 13 15) apparently with a favourable effect but as other treatment has often beed administered concurrently it is difficult to assess the results

The present report concerns a case of an unusual site which was confirmed by biopsy The only treatment given was long term glucocorticoid medication with well-substantiated success

CASE REPORT

The patient was a 53-year-old man who was referred in March 1966 from a medical department for exploratory laparotomy because of a suspicion of pancreatic cancer

The patient's father had died of gout There was no family history of collagen diseases and the patient had not received Deseril

In 1955 and 1965 he had undergone operations for bilateral inguinal hernia During the latter stay in hospital

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unlike the previous films a normal axis for the right kidney and the previous blurring of the right psoas was no longer demonstrable

Follow up eight months after the glucocorticoid medication had been discontinued showed that the patient had maintained his body weight that he was still symptom free and fully capable of working However the ESR and γ-globulin were somewhat elevated The follow up is being continued in case a repeated course of prednisone should be needed

DISCUSSION

The diagnosis in the present case is beyond doubt but the case differs from most of those reported previously in the unusual localization of the fibrosis around the upper pole of the right kidney However there have been previous reports on abnormally situated IRF (4 17) and co-existence of fibrosing mediastinitis and IRF has been described (5)

In this case the steroid therapy is of particular interest There were unmistakable clinical improvement and definite objective criteria of a beneficial effect of the glucocorticoid medication No other treatment was given simultaneously

Steroid therapy has previously been used to a slight extent but as a rule concurrently with other treatment such as surgery (1 5) radiotherapy (8) cytotoxics (8) or various antibiotics (17) Therefore it has been difficult to assess a possible beneficial effect In a very few cases with a histologically confirmed diagnosis steroid therapy has been used alone (2 10 13 15) In cases when the therapeutic result has been followed up there seems to have been a favourable effect of the steroid medication if it is given in high doses over a long period

In our opinion the striking effect in the present case combined with the data given in the literature speaks for long term glucocorticoid therapy in high doses in all cases of IRF also those for which surgery is indicated

REFERENCES

1 Charnock D A Ridell H I & Lombardo L J Retroperitoneal fibrosis producing ureteral obstruction J Urol (Baltimore) 85 451 1961
2 Fischer H R Retroperitoneal fibrosis (Ormond's disease) Helv med Acta 33 44 1966
3 Freestone D S Possible retroperitoneal fibrosis and methysergide Lancet 1 1168 1965
4 Harbrecht P J Variants of retroperitoneal fibrosis *Ann Surg 165 388 1967*
5 Hawk W A & Hazard J B Sclerosing retroperitonitis and sclerosing mediastinitis Amer J clin Path 32 321 1959
6 Johannessen S & Hilden M Idiopatisk retroperitoneal fibrose Nord Med 75 641 1966
7 Lefall L D Retroperitoneal fibrosis—two unusual cases Arch Surg 89 1070 1964
8 Ludwig J & Baumgartner R Die retroperitonaale Fibrose Schweiz med Wschr 93 1405 1963
9 Lund F & Pedersen J Periureteritis fibrosa (Gerota's Fascitis) Acta med scand 167 105 1960
10 Morandi L Camponovo F & Siebenmann R Corticosteroidtherapie bei der chronischen fibrosierenden Retroperitonitis Schweiz med Wschr 93 1409 1963
11 Olsson, S Sjoberg J E Wahlquist L & Zederfeldt, B Idiopatisk retroperitoneal fibrosis Acta chir scand 123 427 1962
12 Oppenheimer G D Narins L & Simon N Radiotherapy in treatment of nonspecific inflammatory retroperitoneal process J Urol (Baltimore) 67 476 1952
13 O Regan R Treaby P A & Prior I A M Idiopathic *retroperitoneal fibrosis A case presenting with* renal failure treated effectively with adrenal steroids N.Z med J 60 518 1961
14 Ormond J K Bilateral ureteral obstruction due to envelopment and compression by an inflammatory retroperitoneal process J Urol (Baltimore) 59 1072, 1948
15 Paper F P Idiopathic retroperitoneal fibrosis involving the ureters Proc roy Soc Med 53 690 1960
16 Reinhard R E Presumptive Ormond s disease Remission following steroid therapy *Henry Ford Hosp Bull* 11 313 1963
17 Schneider C F Idiopathic retroperitoneal fibrosis producing vena caval biliary ureteral and duodenal obstructions Ann Surg 159 316 1964

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ACUTE HEMODYNAMIC CHANGES FOLLOWING BETA ADRENERGIC BLOCKADE IN HYPERTHYROIDISM

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Abst act The acute hemodynamic effects of beta receptor blockade in thyrotoxicosis have been studied in six cases The blockade was effectuated by the intravenous injec tion of 0 1 mg/kg/min of Inderal® (five cases) or Aptin® (one case) In the four cases which at rest showed hyperkinetic circulation there was a substantial decrease in heart rate and cardiac output while the oxygen con sumption was unaltered On the basis of these hemo-dynamic findings which confirm earlier reports as well as some case reports on successful peroral therapy the use of these drugs in thyrotox cosis is recommended Their use is in most cases only temporary until full benef t is derived from measures directly aimed at the thyroid hyperfunction

The hyp rkinetic circulation in hyperthyroidism (26) closely resembles that seen in states of in creased adrenergic activity There is experimental and clinical (10 13 20) evidence of increased sensitivity to catecholamines in induced and spontaneous hyperthyroidism Adrenaline has also been used as a diagnostic test in cases of suspected hyperthyroidism (13 20) The exact mechanism seems however to be unknown and the question is still open whether there is an altered produc tion metabolism or organ sensitivity to catechol amines in hyperthyroidism

The fundamental experimental work by Brewster et al (4) demonstrated that the circulatory effects of induced hyperthyroidism could be abolished by total sympathetic blockade with local anesthetic drugs They also reviewed its clinical use in some early reports dating back to 1936

Sympathicolytic drugs have successfully been used in the treatment of thyrotoxicosis The cir culatory manifestations as well as the lid retraction have been successfully treated with reserpine (8) and guanethidine (12 14 27) The great value of Arfonad® in thyroid crisis has been reported from our clinic (19) It must be stressed that the meta bolic symptoms of thyroid hyperfunction are not influenced by these drugs though there might be some decrease in oxygen consumption and rate of weight loss

During the last few years specific beta adrenergic blocking drugs have become available From a pharmacological point of view these sub stances should be preferred to the sympathicolytic agents in the treatment of circulatory symptoms of thyrotoxicosis Reserpine and guanethidine may cause diarrhea and water retention Reser pine may cause psychic disturbances and guane thidine must be given in very large doses The purpose of this work was to study the immediate effects of beta adrenergic blocking drugs on the central circulation in patients with Grave s disease

MATERIAL

There were five women and one man (Table I) all suffering from hyperthyroidism The diagnosis was verified by careful clinical examination and standard laboratory procedures Sinus rhythm was present in all cases Weight loss was moderate in four pronounced in one and absent in one case All had a goiter as well as a vascular bruit In case E B this murmur was unusually strong with a grade 3 (scale 1–6) continuous murmur audible over the goiter and irradiating down to the fourth intercostal space and high up in the neck An arterio-venous fistula was suspected and a thoraco-cervical aortography performed Th s revealed no shunt but a high contrast dens ty in the thyroid gland

The laboratory find ngs (Table I) reflect the varying degree of severity Thyro dectomy was later successfully performed in four cases The preoperative treatment with thyreostatic drugs was instituted after the examinations to be reported here Thyroid crisis was not seen in this material

METHODS

Right heart catheterization was performed w th standard techn que Catheters were posit oned in the brachial artery

unlike the previous films a normal axis for the right kidney and the previous blurring of the right psoas was no longer demonstrable

Follow up eight months after the glucocorticoid medication had been discontinued showed that the patient had maintained his body weight that he was still symptom free and fully capable of working However the ESR and γ-globulin were somewhat elevated The follow up is being continued in case a repeated course of prednisone should be needed

DISCUSSION

The diagnosis in the present case is beyond doubt but the case differs from most of those reported previously in the unusual localization of the fibrosis around the upper pole of the right kidney However there have been previous reports on abnormally situated IRF (4 17) and co existence of fibrosing mediastinitis and IRF has been described (5)

In this case the steroid therapy is of particular interest There were unmistakable clinical improvement and definite objective criteria of a beneficial effect of the glucocorticoid medication No other treatment was given simultaneously

Steroid therapy has previously been used to a slight extent but as a rule concurrently with other treatment such as surgery (1 5) radiotherapy (8) cytotoxics (8) or various antibiotics (17) Therefore it has been difficult to assess a possible beneficial effect In a very few cases with a histologically confirmed diagnosis steroid therapy has been used alone (2 10 13 15) In cases when the therapeutic result has been followed up there seems to have been a favourable effect of the steroid medication if it is given in high doses over a long period

In our opinion the striking effect in the present case combined with the data given in the literature speaks for long term glucocorticoid therapy in high doses in all cases of IRF also those for which surgery is indicated

REFERENCES

1 Charnock D A Ridell H I & Lombardo L J Retroperitoneal fibrosis producing ureteral obstruction J Urol (Baltimore) 85 251 1961
2 Fischer H R Retroperitoneal fibrosis (Ormond's disease) Helv med Acta 33 44 1966
3 Freestone D S Possible retroperitoneal fibrosis and methysergide Lancet 1 1168 1965
4 Harbrecht, P J Variants of retroperitoneal fibrosis Ann Surg 165 388 1967
5 Hawk W A & Hazard J B Sclerosing retroperitonitis and sclerosing mediastinitis Amer J clin Path 32 321 1959
6 Johannessen S & Hilden M Idiopatisk retroperitoneal fibrose Nord Med 75 641 1966
7 Lefall L D Retroperitoneal fibrosis—two unusual cases Arch Surg 89 1070 1964
8 Ludwig J & Baumgartner R Die retroperitoneale Fibrose Schweiz med Wschr 93 1405 1963
9 Lund F & Pedersen J Periureteritis fibrosa (Gerota's Fascitis) Acta med scand 167 105 1960
10 Morandi L Camponovo F & Siebenmann R Corticosteroidtherapie bei der chronischen fibrosierenden Retroperitonitis Schweiz med Wschr 93 1409 1963
11 Olsson S Sjoberg J E Wahlquist, L & Zederfeldt, B Idiopatisk retroperitoneal fibrosis Acta chir scand 123 427 1962
12 Oppenheimer G D Narins L & Simon N Radiotherapy in treatment of nonspecific inflammatory retroperitoneal process J Urol (Baltimore) 67 476 1952
13 O Regan, R Treahy P A & Prior I A M Idiopathic retroperitoneal fibrosis A case presenting with renal failure treated effectively with adrenal steroids N Z med J 60 518 1961
14 Ormond J K Bilateral ureteral obstruction due to envelopment and compression by an inflammatory retroperitoneal process J Urol (Baltimore) 59 1072, 1948
15 Paper F P Idiopathic retroperitoneal fibrosis involving the ureters Proc roy Soc Med 53 690 1960
16 Reinhard R E Presumptive Ormond's disease Remission following steroid therapy Henry Ford Hosp Bull 11 313 1963
17 Schneider C F Idiopathic retroperitoneal fibrosis producing vena caval biliary ureteral and duodenal obstructions Ann Surg 159 316 1964

Acta med scand Vol 184 pp 235–239 1968

ACUTE HEMODYNAMIC CHANGES FOLLOWING BETA ADRENERGIC BLOCKADE IN HYPERTHYROIDISM

Ingemar Cullhed and Anders Parrow

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Abstract The acute hemodynamic effects of beta receptor blockade in thyrotoxicosis have been studied in six cases The blockade was effectuated by the intravenous injection of 0 1 mg/kg/min of Inderal® (five cases) or Aptin® (one case) In the four cases which at rest showed hyperkinetic circulation there was a substantial decrease in heart rate and cardiac output while the oxygen consumption was unaltered On the basis of these hemodynamic findings which confirm earlier reports as well as some case reports on successful peroral therapy the use of these drugs in thyrotoxicosis is recommended Their use is in most cases only temporary until full benefit is derived from measures directly aimed at the thyroid hyperfunction

The hyperkinetic circulation in hyperthyroidism (26) closely resembles that seen in states of increased adrenergic activity There is experimental and clinical (10 13 20) evidence of increased sensitivity to catecholamines in induced and spontaneous hyperthyroidism Adrenaline has also been used as a diagnostic test in cases of suspected hyperthyroidism (13 20) The exact mechanism seems however to be unknown and the question is still open whether there is an altered production metabolism or organ sensitivity to catecholamines in hyperthyroidism

The fundamental experimental work by Brewster et al (4) demonstrated that the circulatory effects of induced hyperthyroidism could be abolished by total sympathetic blockade with local anesthetic drugs They also reviewed its clinical use in some early reports dating back to 1936

Sympathicolytic drugs have successfully been used in the treatment of thyrotoxicosis The circulatory manifestations as well as the lid retraction have been successfully treated with reserpine (8) and guanethidine (12 14 27) The great value of Arfonad® in thyroid crisis has been reported from our clinic (19) It must be stressed that the metabolic symptoms of thyroid hyperfunction are not influenced by these drugs though there might be some decrease in oxygen consumption and rate of weight loss

During the last few years specific beta-adrenergic blocking drugs have become available. From a pharmacological point of view these substances should be preferred to the sympathicolytic agents in the treatment of circulatory symptoms of thyrotoxicosis Reserpine and guanethidin may cause diarrhea and water retention Reserpine may cause psychic disturbances and guanethidine must be given in very large doses. The purpose of this work was to study the immediate effects of beta adrenergic blocking drugs on the central circulation in patients with Graves disease

MATERIAL

There were five women and one man (Table I) all suffering from hyperthyroidism The diagnosis was verified by careful clinical examination and standard laboratory procedures Sinus rhythm was present in all cases Weight loss was moderate in four pronounced in one and absent in one case All had a goiter as well as a vascular bruit In case E B this murmur was unusually strong, with a grade 3 (scale 1–6) continuous murmur audible over the goiter and irradiating down to the fourth intercostal space and high up in the neck An arterio-venous fistula was suspected and a thoraco-cervical aortography performed. This revealed no shunt but a high contrast density in the thyroid gland

The laboratory findings (Table I) reflect the varying degree of severity Thyroidectomy was later successfully performed in four cases The preoperative treatment with thyreostatic drugs was instituted after the examination to be reported here Thyroid crisis was not seen in the material

METHOD

Right heart catheterization was ... technique Catheters were positi...

Table I *Clinical and laboratory findings*

Case	Age	Sex	Weight loss	Struma	Exopht.	Bruit	BMR ( )	PBI (μg/100 ml)	TIT uptake ( )	I^{131} uptake (1 h %)
S D	36	♀	+	+	0	(+)	+48	13 7	38 9	37 5
H F	52	♀	+	+	+	+	+46	15 3	—	30 4
M H	40	♀	+ +	+	+	+	+52	9 7	25 5	—
E B	36	♀	0	+	+	+ +	+31	7 3	31 9	81 8
I T	56	♀	+	+	+	+	+54	14 0	37 8	23 8
B B	30	♀	+	+	0	+	+47	—	35 5	—

BMR = basal metabolic rate PBI = protein bound serum iodine TIT = triiodothyronine uptake in red blood cells I^{131} = uptake in thyroid gland during the first hour
(+) = questionable + = moderate + + = marked

and the pulmonary artery After 10 min rest the cardiac output was determined according to Fick's principle Oxygen consumption was measured during 10 min. In the middle of this period blood samples were drawn and pressures registered. The active drug was then administered, with 0 1 mg kg body weight/min. In five cases propranolol (Inderal®) was injected in one case H 56/28 (*dl* 1-(o-allyl phenoxy)-3-isopropylamino-2 propanol hydrochloride Aptin® (3)) in the same dose The drugs were given via the catheter into the pulmonary artery Ten min after the injection the cardiac output and the pressures were again measured

In cases E B and B B studies were made at rest as well as during supine exercise on an electrically braked bicycle ergometer In case I T cardiac output was measured at rest, 10 min after a saline injection and then 10 min after injection of the active agent.

RESULTS

These are presented in Table II Tachycardia was presented in only three cases In four cases the arterio-venous oxygen difference (AVD) was low 28 4–34 5 ml/l with an increased cardiac index, 5 1–6 3 l/min/sqm The two cases with a normal

Table II *Hemodynamic findings*

Case	Age	Period	Heart rate	V_{O_2} (ml/min)	AVD (ml/l)	CO (l/min)	CI (l/min/m²)	SV (ml)	R_S (u)	R_P (u)
S D	36	Rest, before	116	276	31 3	8 8	6 3	76	—	1.2
		Rest, after 4 mg Inderal	83	[illegible]66	39 6	6 7	4 8	81	—	1 4
H F	52	Rest, before	84	345	42.1	8 [illegible]	4 4	98	10	1 8
		Rest, after 7 mg Aptin	81	3[illegible]6	43 9	7 4	4 0	9[illegible]	11	—
M H	40	Rest, before	82	245	42 3	5 8	4 0	71	17	1 0
		Rest, after 5 mg Inderal	80	24[illegible]	37 7	6 4	4 4	80	16	0 5
E B	36	Rest, before	85	249	[illegible]8 4	8 8	5 1	104	11	1 7
		Work, 300 kpm min	1[illegible]8	891	56 6	15 7	9 1	122	8	1 4
		Rest, after 6 mg Inderal	65	216	41 0	5 3	3 1	81	16	3 6
I T	56	Rest, before	100	277	33 3	8 3	5 4	83	1	1 6
		Rest, saline	99	266	33 1	8 0	5 2	81	11	1 9
		Rest after 5 mg Inderal	88	263	39 4	6 7	4 4	76	15	[illegible]5
B B	30	Rest, before	116	275	34 5	8 0	5 8	69	12	1 1
		Work, 250 kpm/min	179	112[illegible]	93 8	12.0	8 7	67	11	1 8
		Rest after 5 mg Inderal	101	286	43 1	6 6	4 8	66	14	—
		Work 250 kpm/min	133	97[illegible]	103 8	9 4	6 8	71	13	2.0

V_{O_2} = oxygen uptake. AVD = arterio-venous oxygen difference CO = cardiac output CI = cardiac index SV = stroke volume R_S = systemic arterial resistance R_P = pulmonary arterial resistance

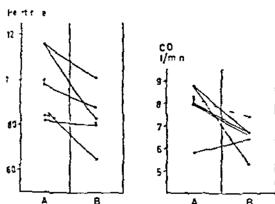


Fig 1 Heart rate and cardiac output at rest before (A) and after (B) beta adrenergic blockade Straight lines represent cases with Inderal® interrupted line the case with Apun® The short vertical line in A represents the effect of placebo (saline) injection

AVD had a cardiac index within the upper normal range (4 0–4 4 l/min/sqm)

In two case cardiac output was determined during exercise In case E B there was an exaggerated increase in cardiac output in relation to the increase in oxygen consumption while the increase in cardiac output in case B B followed the normal regression line

After beta receptor blockade there was a decreas in heart rate and cardiac output in only those four cases who had a hyperkinetic circulation before (Table II Fig 1) This was caused by an increase in AVD though in case E B the oxygen consumption also fell In this case there was a substantial fall in stroke volume The two cases with normal AVD and heart rate showed no significant changes in heart rate or cardiac index

The injection of saline in case I T which was performed in exactly the same way as the injection of the active drug caused no significant changes in any studied parameter

In one case the effects of propranolol were studied at rest as well as during exercise (Table II Fig 2) Before the injection of propranolol the heart rate rose to high values at exercise and the patient became nearly exhausted After Inderal® the heart rate rose much less on the same work load and the patient tolerated the work test better

There were only small changes in vascular resistances (Table II) No side-effects were noted

DISCUSSION

The sympathicomimetic inotropic and chronotropic actions on the heart are mediated via the beta adrenergic receptors Several drugs with a more or less specific blocking effect on these receptors have been synthesized In 1964 Wilson et al (28) reported their findings with nethalide in 11 cases with spontaneous hyperthyroidism In a later study from the same group (29) the effects of 0 15 mg/kg Inderal® on induced hyperthyroidism in healthy subjects were reported Both drugs inhibited the effects of isoprenaline infusion but the basal heart rate and cardiac output was not influenced They concluded that the circulatory effects of spontaneous hyperthyroidism and induced hypermetabolism were not mediated through stimulation of beta adrenergic receptors

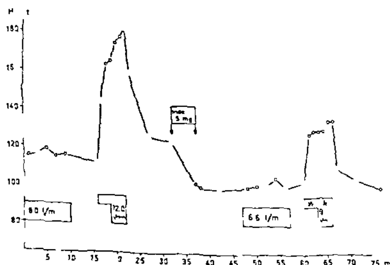


Fig Heart rate in case B B at rest and exercise (00 kpm/min) before and after the injection of Inderal® Rectangles with text *Inderal* and *Work* indicate the time for injection and exercise respectively Rectangles with values in l min represent cardiac output and the time for its determination

and 184

A favorable influence of Inderal® on the tachycardia of anxiety states has been reported (2 15 21 24) In thyrotoxicosis a marked in some cases dramatic effect on heart rate was found when Inderal® was given in a single dose intravenously (5 mg) or by mouth (30 mg) (2 24) The clinical effect of Inderal® in more prolonged peroral therapy in cases with severe thyrotoxicosis has been reported (5 22) There was a very good effect on tachycardia, but also on other symptoms such as rapidity of weight loss nervousness tremor and lid retraction. As with reserpine and guanthidine the thyroid hyperfunction was not influenced Recently two cases with thyroid crisis were advantageously treated with peroral Inderal® (6)

The hemodynamic response to a single intravenous injection of Inderal® was studied by Howitt and Rowlands 1966 (17) They injected 15 mg in 20 cases of Grave s disease In all cases there was a significant decrease in heart rate at rest as well as during exercise while the cardiac output decreased in all but two cases In our material the heart rate also decreased, though in ly in two cases. We used a smaller dose which might not have resulted in an effective receptor blockade in all cases However no further changes were noted if 5 mg of Inderal® were added to a first dose of 10 mg (17) It has been found that Inderal® in a dose of 0 1 mg/kg causes a virtually complete beta receptor blockade in man (16) However after 5–10 mg Inderal® intravenously isoprenaline will give a slight increase in heart rate but not in cardiac output (9)

The absence of response to Aptin® in one case could be due to the fact that this drug has a weak beta receptor stimulating action (1) However in the doses used (5 mg) Inderal® and Aptin® were found to be equipotential when the effect of isoprenaline infusion (0 09 µg/kg min) was studied (18) Further the case where Aptin® was given without demonstrable effect had a normal heart rate and cardiac output at rest. The same applies to the other case in which Ind ral® was given without significant reaction. It must be admitted that a higher dose could have elicited a more pronounced effect. However even with the relatively small doses employed in this study a clear effect was seen in four cases out of six, which were also those with the most pronounced hyperkinetic circulation.

The effect of Inderal® on the circulatory response to exercise is a decreased rise in heart rate and cardiac output compared with the changes at work before blockade (9 11 23 25) These findings can be illustrated by Fig 2. The lower values at rest after the first exercise period could be an effect of exercise itself and this applies also to the lower cardiac output during the second work test. However these changes are probably more significant after work in sitting position than after supine work (7)

REFERENCES

1 Ablad, B., Brogard, M & Ek, L. Pharmacological properties of H 56/28—a β adrenergic receptor antagonist Acta pharmacol (Kbh.) Suppl. 2 9 1967

2 Bollinger A., Gander M & Forster G., Pulsfrequenz und Leistungsfähigkeit vor und nach β Rezeptoren Blockade durch Propranolol. Schweiz. med. Wschr 95 1075 1965

3 Brandstrom, A. Corrodi, H., Junggren, U & Jonsson, T E.. Synthesis of some β adrenergic blocking agents Acta Pharm. Suecica 3 303 1966

4 Brewster W R., Isaacs, J P., Osgood P F & King, T L. The hemodynamic and metabolic interrelationsships in the activity of epinephrine norepinephrine and the thyroid hormones Circulation 13 1 19 6

5 Buckfield, P M & Davis, J A. β adrenergic blockers in childhood thyrotoxicosis Lancet 1 14_5 1966

6 Buckle R. M. Treatment of thyroid crisis by beta adrenergic blockade Acta endocr (Kbh) 57 168 1968

7 Burkart, F Barold, S & Sowton, E. Hemodynamic effects of repeated exercise Amer J Cardiol 20 509 1967

8 Canary J J., Schaaf, M Duffy B J & Kyle L H. Effects of oral and intramuscular administration of reserpine in thyrotoxicosis New Engl J Med. _57 435 1957

9 Cumming, G R. & Carr W Hemodynamic response to exercise after propranolol in normal subjects. Canad. J Physiol. Pharmacol. 44 465 1966

10 Danowski, T S Heineman, A. C Bonessi, J V & Moses C.. Effects of thyroid hormone excesses on pressor activity and epinephrine responses Metabolism 13 747 1964

11 Epstein, S E Robinson, B F Kahler R. L & Braunwald, E Effects of beta adrenergic blockade on the cardiac response to maximal and submaximal exercise in man. J clin. Invest. 44 1745 1965

1_ Gaffney T E Braunwald, E. & Kahler R. L.. Effects of guanethidine on tri-iodothyronine-induced hyperthyroidism in man New Engl J Med _65 16 1961

13 Goethsch, E Newer methods in the diagnosis of thyroid disorders N Y St. J Med. 18 _9 1918

14 Goldstein, S & Killip T Catecholamin depletion in thyrotoxicosis Circulation 31 _19 1965

15 Granville-Grossman, K. L & Turner P Th. effect of propranolol on anxiety Lancet 1 783 1966.

16 Harrison D C & Griffin J R Metabolic and circulatory responses to selective stimulation and blockade of the β adrenergic system Clin Res 13 09 1965
17 Howitt, G & Rowlands D J Beta sympathetic blockade in hyperthyroidism Lancet 1 628 1966
18 Johnsson G Norrby A & Solvell L Potency and time-effect relationship in man of propranolol and H 56/?8 Acta pharmacol (Kbh) Suppl 2 1967
19 Malers E Werner I & Lof B Trimetaphan camphor-sulphonate in the treatment of postoperative thyroid crisis Acta med scand 169 551 1961
0 Murray J F & Kelly J J The relation of thyroid hormone level to epinephrine response a diagnostic test for hyperthyroidism Ann intern Med 51 309 1959
1 Nordenfelt O Orthostatic ECG changes and the adrenergic beta receptor blocking agent propranolol *Acta med scand 178 4 1965*
22 Robillard M Klots B & Perrault M L inhibition des récepteurs beta adrenerg ques dans le traitement de la maladie de Basedow Presse méd 75 897 1967
.3 Sowton E & Hamer J Hemodynam hang af r beta adrenergic blockade Amer J C ol 18 1966
?4 Turner P Granville Grossman K L & Sm Effect of adrenergic receptor blo k o th a f cardia of thyrotoxicosis and anxi t e L nc 1316 1965
?5 Vogel J H K & Blount S G M d c cardiovascular responses by propranolol B H .9 310 1967
.6 Wade O L & Bishop J M Cardia regional blood flow p 19 Blackwell Oxf 1
27 Waldstein S S West G H Lee W Y S B D Guanethidine in hyperthyroidism J Am r Ass 189 609 1964
28 Wilson W R Theilen E O & Fletcher F Pharmacodynamic effects of beta adrene gi re blockade in patients with hyperthyroidism J Invest *43 1697 1964*
29 Wilson W R Theilen E O Hege J H & V len M R Effects of beta adrenerg c receptor b ock in normal subjects before during and afte iodothyronine induced hypermetabolism J clin Inve 54 1159 1966

Congress Announcements

The Second International Meeting of the International Society for Neurochemistry (ISN) will be held in Milan Italy September 1 to 5 1969

Secretaries

For details and registration forms contact Dr J Folch Pi Dept of Neurochemistry McLeans Hospital Harvard Medical School Belmont Mass 02178 USA or Dr Rodolfo Paoletti Institute of Pharmacology University of Milan Via Andrea del Sarto 21 20129 Milan Italy

The Fourth International Congress on Hyperbaric Medicine will be held at the Park Hotel Sapporo Japan September 2 to 4 1969

President Juro Wada M D

Secretary General Takashi Iwa M D

Secretaries Koji Ikeda M D and Dorothy J Boone Dept of Thoracic and Cardiovascular Surgery Sapporo Medical College South 1 West 16 Sapporo Japan

The Tenth International Cancer Congress with four preliminary special sessions will be held in Houston under the auspices of the International Union Against Cancer (UICC) May 22 to 29 1970

Information from National Organizing Committee Secretariat The University of Texas M D Anderson Hospital and Tumor Institute 6723 Bertner Avenue P O Box 20465 Astrodome Station Houston Texas 77025

Chairman R Lee Clark M D

Secretary General Murray M Copeland M D

International Congress of Radiology in the Netherlands

The European Association of Radiology will hold its 2nd Congress in the Netherlands Congress Centre at The Hague June 14 to 18 1971

The organization has been entrusted to the Netherlands Association of Radiology

President of the Congress Professor J R von Ronnen of Leiden University

Approximately 2000 participants are expected to attend Information from the local secretariat c/o Holland Organizing Centre 16 Lange Voorhout The Hague

Acta med scand Vol 184 pp 241–246 1968

THE PLASMA LIPIDS AND THEIR FATTY ACID PATTERN IN MYOCARDIAL INFARCTION

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Abstract The concentrations of total plasma lipid the major plasma lipid fractions including cholesterol, triglycerides and phospholipids and the fatty acid composition of the lipid fractions mentioned were determined partly by gas liquid chromatography in 21 male and 11 female patients suffering from acute myocardial infarction during the first 1–2 days of their disease and—in the survivors—4 to 12 months later The results were compared with those of 18 male and 10 female normal persons without any known coronary disease and whose age was comparable with that of the patients

In most cases the results for the acute patients did not differ significantly from those for the controls The analyses of the re-examined patients showed increased concentrations of plasma total lipid and cholesterol as compared with those of the controls

The fatty acid composition of the main plasma lipid fractions was not significantly changed in the two patient groups as compared with that of the controls with one exception very low or zero values of linolenic acid of the plasma lipid fraction were significantly more frequent in the acute patients as compared with the controls

The importance of lipids in the pathogenesis of atherosclerosis is still controversial From some prospective studies it appears however that the risk of experiencing a clinical episode of coronary disease is amongst others a function of plasma cholesterol level (11 17 23)

With the advance of gas liquid chromatography much attention has been paid to determination of the fatty acid patterns of the different lipid fractions in human blood Whereas this technique is too laborious and time consuming for prospective investigations only a few studies have compared the composition of lipids in sera of normal persons and of patients with coronary arterial disease (4 5 9 13 15 22 28) By and large only few differences have been observed

The present paper is a report on a study of the major plasma lipid fractions and their fatty acid patterns from C14 0 through C20 4 in apparently healthy controls and in patients with acute myocardial infarction As far as it was possible the patients were examined twice in most cases initially within the first two days after the actual attack and subsequently between two and twelve months later Thanks to a modification of the technique applied it has been possible to obtain a fairly accurate estimate of the fatty acids which are but scantily represented

MATERIAL AND METHODS

The material comprises 21 male and 11 female patients mean age 64 years (range 40–91 years) and 66 years (range 55–76 years) respectively All the patients had unequivocal myocardial infarction according to the conventional criteria

On the first morning after admission within 8–7 hours average 30 hours after onset of the acute attack, blood specimens were drawn from the fasting patient (patient group I) This examination was repeated for 13 of the male patients representing all the survivors between 4 and 12 months later on an average 6 months and for the 6 surviving female patients between and 13 months later average 8 months (patient group II)

The control material consisted of subjects without any known coronary or other atherosclerotic disease (controls) This material comprised 18 men, mean age 5. years (range 3 to 69 years) and 10 women, mean age 48 years (range 35 to 62 years) Blood specimens were drawn in a state of fasting

The different analytical methods for lipid assay and their respective accuracy have been described previously (1) The gas liquid chromatographic recorder was provided with an automatic attenuator This device allowed relatively large amounts of lipid material (0 05–0 1 microg) to be put on the column Consequently the peaks of the minor fatty acid components could be better represented than is usually the case whereas the peaks of the major components could be kept within suitable limits by automatically reducing the sensitivity of the recorder from one to several times by a factor two (Fig 1)

The gas liquid chromatographic results were obtained

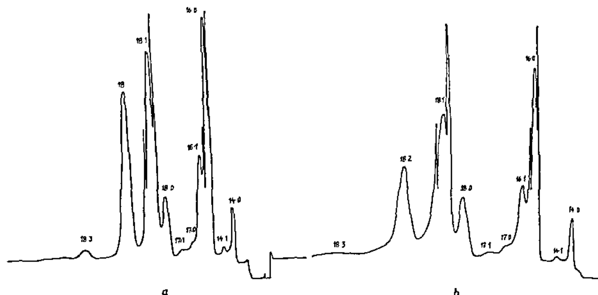


Fig 1 Gas liquid chromatogram of the fatty acids of plasma triglyceride from a control (*a*) and a patient (group I) (*b*) The difference in the concentration of linolenic acid is remarkable

as a percentage of the fatty acids present in the different plasma lipid fractions cholesterol esters triglycerides and phospholipids The chromatographic analysis of the non-esterified fatty acid (NEFA) was omitted as this fraction is quantitatively insignificant

RESULTS

The mean values $\pm$ S D obtained for the major plasma lipid fractions in patient groups I and II are compared mutually and with those of the controls in Table I Statistical analyses (Student s *t* test) were carried out to detect any significant differences between the three groups studied Regarding the controls versus male patient group I no such differences were found In female patient group I the total lipids and the triglycerides were significantly higher than those of the controls In comparison with the controls both sexes in patient group II had significantly higher values for total lipids and total cholesterol The same applied to cholesterol esters in the female patients During the course of the disease—comparing patient group I with group II—total lipids total cholesterol and phosphatides increased significantly in the male patients The serum triglycerides decreased in both sexes but significantly so in the females only

Table I *Concentration of plasma total lipids cholesterol cholesterol esters triglycerides and phosphatides in controls and in patient groups I and II*

d = difference between mean values signific = statistical significance of difference n s = non significant *p* = probability

	Sex	Controls	Patient group I	Patient group II	Patient group I—controls		Patient group II—controls		Patient group I—patient group II	
					d	Signific	*d*	Signific	*d*	Signific
Total plasma lipids	♀	*618± 86*	*774±211*	*772±124*	*156*	−($p<0\ 05$)	*154*	+($p<0\ 02$)	2	n s
	o	666± 84	678±138	793±116	12	n s	1_7	+($p<0\ 001$)	94	+($p<0\ 05$)
Plasma cholesterol	♀	209± 36	234± 57	276± 53	25	n s	67	+($p<0\ 02$)	42	n s
	♂	211± 29	215± 62	279± 36	4	n.s	68	+($p<0\ 001$)	62	+($p<0\ 001$)
Plasma cholesterol esters	♀	249± 41	296±112	333±173	47	n s	87	+($p<0\ 01$)	40	n s.
	o	255±133	272± 71	299± 56	17	n s	44	n s	_7	n s
Plasma triglycerides	♀	78± 37	182± 81	10_± 12	104	+($p<0\ 01$)	24	n s	80	+($p<0\ 05$)
	♂	144± 37	144± 56	123± 70	0	n s	21	n s	21	n s
Plasma phosphatides	♀	230± 23	258± 73	265± 43	28	n s	35	n s	7	n s.
	♂	234± 40	225± 47	259± 38	9	n s	25	n s	34	+($p<0\ 0\ $)

Table II *Fatty acids of the plasma cholesterol esters as per cent*

Fatty acids	Sex	Controls	Patient group I	Patient group II	Patient group I—controls		Patient group II—controls		Patient group I—patient group II	
					d	Signific	*d*	Signific	*d*	Signific
C14 0	♀	0 5±0 31	0 2±0 2[illegible]	0 3±0 37	0 3	n s.	0 2	n s	0 1	n s
C16 0		12 2±2 72	10 7±2 01	13 5±3 30	1 5	n s	1 3	n s	2 8	n s
C16 1		4.2±1 48	4 7±1 61	6 3±0 81	0 5	n s.	2 1	n s	1 6	n s
C18 0		0 7±0 22	0 6±0 24	0 5±0 71	0 1	n s	0 2	n s.	0 1	n s
C18 1		18 9±2.64	22 9±3 28	22.5±3 51	4 0	n s	3 6	n s	0 4	n s
C18 2		51 0±4 68	49 2±8 80	46 6±3 33	1 8	n s	4 4	n s	2 6	n s
C18 3		0 4±0 38	0 2±0 35	0 3±0 3	0 2	n.s	0 1	n s.	0 1	n s
C20 4		4 8±0 58	5 3±1 69	4 3±1 95	0 5	n s	0 5	n s	1 0	n s
C14 0	♂	0 4±0 24	0 4±0 36	0 4±0 23	0	n s	0	n s	0	n s
C16 0		12.5±2.50	10 9±2.34	12 1±1 03	1 6	n s	0 4	n.s	1 2	n s
C16 1		4 2±0 97	4 4±1 88	5 6±2 11	0 2	n.s	1 4	n.s	1 2	n s
C18 0		1.2±0 69	0 6±0 38	0 6±0 22	0 6	n s	0 6	n s	0	n s
C18 1		25 2±6 70	22 6±3 86	22 4±3 39	2.6	n s.	0 8	n s	0 2	n s
C18 2		45 9±6 69	48 9±7 27	49 1±4 53	3 0	n s	3.2	n s	0 2	n s
C18 3		0 5±0 37	0 2±0 34	0 3±0 34	0 3	n s	0 2	n s	0 1	n s
C20 4		4 1±1 75	4 0±1 23	4 5±1 11	0 1	n s	0 4	n s.	0 5	n s

Abbreviations see Table I

The fatty acid patterns of the three major lipid fractions in the controls and in the two groups of patients are shown in Tables II–IV The peaks recorded for C20 0 C20 1 C20 2 and C20 3 were in ea h case too low to permit calculation The values for linolenic acid (C18 3) will be further dealt with separately in Table V The values obtained ±s D for the different fatty acids were subjected to statistical analyses to detect any significant differences between these values in the controls and in the patients (groups I and II) suffering from coronary infarction No such differences were observed

The frequency of extremely low values ob tained for linolenic acid (C18 3) is given in Table V The zero values include extremely low values inaccessible for computation In both groups of patients of both sexes the incidence of such low values was far higher than in the con trols However this difference has been shown to be significant only for patients in group I (both sexes)

Table III *Fatty acids of the plasma triglycerides as per cent*

Fatty acids	Sex	Controls	Patient group I	Patient group II	Patient group I—controls		Patient group II—controls		Patient group I—patient group II	
					d	Signific	*d*	Signific	*d*	Signific
C14 0	♀	1 8±0 81	0 5±0 14	1 3±1 06	1 3	n s	0 5	n s	0 8	n s
C16 0		27 3±3 22	27 0±5 52	25 7±3 38	0 3	n s	1 6	n s	1 3	n s
C16 1		5 2±2 56	8 6±2 01	10 0±2.92	3 4	n s.	4 8	n s	1 4	n.s
C18 0		5 5±1 70	5 4±1 52	4 4±0 78	0 1	n s	1 1	n s	1 0	n s
C18 1		35 5± 42	40 8±4 75	43 1±3 73	5 3	n s	7 6	n.s	2.3	n s
C18 2		14 5±2 63	14 2±3 5	12 7±5 10	0 3	n s	1 8	n.s	1 5	n s.
C18 3		0 7±0 8	0 3±0 32	0 6±0 47	0 4	n s	0 1	n s	0 3	n s.
C14 0	♂	1 4±0 59	0 7±0 56	0 2±1 06	0 7	n s	1	n s	0 5	n s
C16 0		9 4±2.74	27 5±4 58	30 1±4.25	1 9	n s.	0 7	n s	2.6	n s
C16 1		5 7±1 46	6 9±2.15	8 6±1 53	1 2	n s	2 9	n s	1 7	n s
C18 0		6 1±2 75	5 0±1 [illegible]6	4 9±1 12	1 1	n s.	1 [illegible]	n.s	0 1	n s
C18 1		42.4±6 [illegible]4	43 1±4 07	39 4±3 0	0 7	n s	3 0	n s	3 7	n s
C18 2		11 3±2.53	13 0±3 09	13 2±3 79	1 7	n s	1 9	n.s.	0 2	n.s
C18 3		0 9±0 33	0 5±0 37	0 8±0 37	0 4	n.s	0 1	n s	0 3	n.s

Abbreviations see Table I

Table IV *Fatty acids of the plasma phospholipids as per cent*

Fatty acids	Sex	Controls	Patient group I	Patient group II	Patient group I—controls		Patient group II—controls		Patient group I—patient group II	
					d	Signific	*d*	Signific	*d*	Signific
C14 0	♀	0 2±0 11	0	0	0 2	n s.	0 2	n s.	0	
C16 0		30 0±1 87	29 0±1 05	33 4±2 71	1 0	n s.	3 4	n.s.	4 4	n s.
C16 1			1 3±0 53							
C18 0		16 4±1 45	15 1±2 69	17 0±1 70	1 3	n s.	0 6	n s.	1 9	n.s.
C18 1		13 9±2 42	18 6±2 05	15 8±2 51	4 7	n s.	1 9	n.s	2.8	n s.
C18 2		22 5±. 91	19 8±2 63	20 7±3 85	2 7	n s.	1 8	n s.	0 9	n s.
C18 3		0 4±0 11	0 3±0 30	0 3±0 29	0 1	n s	0 1	n s.	0	n s.
C20 4		8 6±1 35	8 6±1 74	7 0±1 55	0	n.s	1 6	n s.	1 6	n s.
C14 0	♂	0 2±0 13	0 1±0 15	0 1±0 ?1	0 1	n s	0 1	n s.	0	
C16 0		30 7±5 06	33 1±6 08	32.3±4 30	2 4	n s	1 6	n s	0 8	n s.
C16 1		1 2±0 57								
C18 0		17 5±3 63	15 3±2 93	16 5±1.26	2 2	n s	1 0	n s	1 .	n.s.
C18 1		16 1±2 69	15 9±2.38	16 0±2 78	0 2	n s	0 1	n s	0 1	n s.
C18 2		19 9±4 38	20 8±5 11	2..6±3 38	0 9	n s	2 7	n s.	1 6	n s.
C18 3		0 5±0 28	0 3±0 35	0 5±0 41	0 2	n s	0	n s.	0 2	n s.
C.0 4		7 6±2 07	7 5±2 03	7 4±1 86	0.1	n s	0 2	n s.	0 1	n s.

Abbreviations see Table I

DISCUSSION

As regards the major plasma lipid fractions the mean values recorded for the male controls are in fair accordance with those observed in larger materials from the more prosperous Western countries (2 14 22) As far as we are aware extensive studies of healthy females are lacking In the present female controls however the plasma concentration of triglycerides is on an average remarkably low as compared with that of the males whereas there is a close agreement with respect to the concentrations of cholesterol and phospholipids in the two sexes

Admittedly the controls were all apparently healthy but being middle aged only few of them —if any—were free from atherosclerosis. It would thus be fallacious to consider the controls biochemically normal or even optimal (24 27)

A study of Table I will show that in the male *patients with recent coronary infarction* (group I) the major lipid fractions are practically identical with those of the controls On the other hand the corresponding female patients have significantly higher values for triglycerides than their controls On re-examination (patient group II) both sexes were very much alike Their total cholesterol was now significantly increased in comparison with the controls and the original remarkable elevation of triglycerides was largely eliminated in the females

It has been shown repeatedly that acute myocardial infarction is followed by marked changes in the plasma lipids This applies to cholesterol (3 7 16) plasma lipoproteins (7 16) and to all the major plasma lipid fractions (26) The latter investigation is especially pertinent to the present study According to this the changes in cholesterol and phospholipids were uniform Both decreased significantly during the first week after infarction but regained the initial level after a further two weeks Subsequently no substantial changes were recorded The triglycerides rose markedly from the second day culminating after three weeks Here the initial level was not reached

Table V *Frequency (in per cent) of values of ero of linolenic acid in the main plasma lipid fractions in controls and patient groups I and II*

	Sex	Cholesterol esters (of zero)	Triglycerides (of zero)	Phospholipids (of zero)
Controls	♂	36 4	0	0
Patient group I		80 0	40 0	40 0
Patient group II		50 0	33 3	42 8
Controls	♀	.2	0	10 0
Patient group I		60 0	35 0	50 0
Patient group II		38 5	7 7	23 1

until after about twelve months The present "normal cholesterol values reflect in all probability the usual initial decrease in this lipid The rather high cholesterol values recorded on re examination are more likely to be representative of the pre infarction level This assumption must however be based on experiences from other studies (27)

Some evidence emphasizes the importance of triglycerides in the genesis of myocardial infarction (6 8 18 19) Whereas the findings for the male patients in the present investigation do not lend support to this hypothesis the observations in the females are at first sight more suggestive They involve the low triglyceride values in the female controls as compared with those of the males and the high values recorded shortly after the acute attack The latter phenomenon may however simply be an expression of the above mentioned rise in this lipid immediately following myocardial infarction On re-examination triglyceride values in female patients and controls do not differ significantly

Concerning the fatty acid composition of the major lipid fractions a study of Tables II and IV corroborates the well known considerable difference in the fatty acid pattern of the triglycerides cholesterol esters and phospholipids As regards this pattern there are no significant differences between the two sexes Furthermore it is notable that the fatty acid pattern of the two groups of patients closely resembles that of the controls Several authors have reported that the serum cholesterol esters of subjects with atherosclerosis contain a lower percentage of linoleic acid (C18 2) than that of healthy subjects (4 5 9 15 22 28) The present findings are however in accordance with the observations of Lawrie et al (13) who did not observe any differences in the cholesterol ester fatty acid composition between healthy sub ects and patients with ischaemic heart disease As regards the fatty acid patterns of triglycerides and phospholipids it is generally accepted that they are not influenced by atherosclerotic disease (13 22 25)

In 1964 Owren et al (20) stated that the polyunsaturated fatty acid linolenic acid (C18 3) should be regarded as having an almost specific significance for thrombocyte adhesiveness Increased platelet adhesiveness which is considered of importance for a tendency to thrombosis and which is regularly found in atherosclerosis diabetes and other conditions should be reduced by adding linseed oil or linolenic acid to the food In 1965 however Owren et al (21) admitted that the described effect of linolenic acid in decreasing platelet adhesiveness could not be reproduced in patients with coronary heart disease or other arteriosclerotic diseases

After the publication of Owren s first paper it was considered worth while to subject the minor fatty acids to a more detailed examination than hitherto The result of this investigation has been the observation of a remarkably high frequency of very low or zero concentrations of the linolenic acid in the three major plasma lipid fractions presented in Table IV In relation to coronary heart disease it is so far not possible however to attribute any pathogenetic significance to linolenic acid or rather to its abscence

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REFERENCES

1 Bang, H O Hess Thaysen, E & Gammelgaard F Dan med Bull 14 27 1967
2 Berge A & Nicolaysen, R Scand J clin Lab Invest 15 ₄84 1963
3 Biorck G Blomqvist G & Sievers J Acta med scand 156 493 1957
4 Bjorntorp P & Hood B Circulat Res 8 319 1960
5 Bottcher C J F & Woodford F P J Atheroscler Res 1 434 1961
6 Carlson L A Acta med scand 167 399 1960
7 Dodds C & Mills G L Lancet 1 1160 1959
8 Havel R J & Carlson L A Metabolism 11 195 196
9 James A T Lovelock, J E Webb J & Trotter W R Lancet I 705 1957
10 Jervell A Meyer K & Westlund K Acta med scand 177 13 1965
11 Kannel W B Dawber T R Kagan A Revotskie N & Stokes J Ann intern Med 55 33 1961
1 Keys A Michelsen O Miller E O Hayes E R & Todd R L J clin Invest 9 1347 1950
13 Lawrie T D V McAlpine S G Rifkind B M & Robinson J F Lancet 1 4 1 1961
14 Lawry E Y Mann G V Peterson A Wysocki A P O Connel R & Stare F J Amer J Med ₋ 605 1957
15 Lewis B Lancet 71 1958
16 Little A Shanoff H M Roe R D & Csima A Circulation 31 854 1965
17 Morris, J N Kagan, A Pattison D C Gardner M J & Raffle P A B Lancet 553 1966

18 Nicolaysen, R. & Westlund K.. Scand. J clin. Lab Invest. 15 299 1963
19 — Scand J clin. Lab Invest. 15 291 1963
20 Owren, P A., Hellem, A. J & Ødegård A. Lancet 2 975 1964
21 — Lancet 2. 849 1965
22. Schrade W., Biegler R. & Bohle E.. J Atheroscler Res 1 47 1961
23 Stamler J Conn Med 28 675 1964
24 Stamler J., Berkson, D M Lindberg, H A., Hall, Y., Miller W Majonnier L., Levinson, M., Cohen, D B & Young, Q D Med Clin. N Amer 50 229 1966
25 Swell L Schools, P E., Jr & Treadwell C R. Proc. Soc exp Biol. (N Y) 109 682, 1962.
26 Tibblin, G & Cramer K. Acta med. scand. 174 451 1963
27 Westlund K. Bibl "Nutr et Dieta" (Basel) 6 32, 1964
28 Wright, A. S Pitt, G A. J & Morton, R. A. Lancet 2 594 1959

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ORAL TREATMENT OF PERNICIOUS ANEMIA WITH HIGH DOSES OF VITAMIN B_{12} WITHOUT INTRINSIC FACTOR

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Abstract The concluded results of an experimental study are reported confirming that the absorption of vitamin B_{12} takes place in two ways 1 through the mediation of intrinsic factor 2 without mediation of intrinsic factor The former mechanism allows a B_{12} absorption in normally IF sensitive cases of pernicious anemia and in normal persons with an upper limit of about 2 μg The latter mechanism allows an absorption of vitamin B_{12} which is roughly proportional to the oral dose administered and amounts to about 1 2 % of the dose within a very wide dose range Thus it is possible to increase the uptake to any desired level This direct uptake was found to be of the same magnitude irrespective of whether the patient had a normal absorption was suffering from pernicious anemia with or without resistance to intrinsic factor preparations or had some other form of disturbed B_{12} absorption (idiopathic malabsorption postoperative state after extensive gastric or intestinal resections or ileitis) The result of a long term clinical trial is also reported in which a daily oral dose of 500–1000 μg of vitamin B_{12} without intrinsic factor was given to patients with pernicious anemia and other types of vitamin B_{12} deficiency The material comprised 64 patients followed for up to more than 5 years After the remission period very few serum B_{12} determinations showed borderline or subnormal values and low levels were never of long duration At the conclusion of the study the serum B_{12} values were well within the normal range in all cases Also the individual mean values of the B_{12} determinations made during the observation period from the second month of treatment were normal in all cases No neurological complications were observed The blood values were normal in all but a few cases in which concurrent diseases were present (malignancy chronic infections iron deficiency states) Experimental evidence obtained indicates that oral treatment with 500 μg of B_{12} daily has also resulted in replenishment of the B_{12} depots The treatment with high oral doses of vitamin B_{12} thus constitutes a convenient and completely reliable maintenance therapy in pernicious anemia and other states of B_{12} deficiency and is a fully acceptable alternative to the conventional method of vitamin B_{12} injections This type of therapy (1000 μg B_{12} daily) was introduced into Sweden in 1964 and is now extensively used

During recent years many reports have been published suggesting that high doses of vitamin B_{12} alone administered orally can produce a satisfactory effect on pernicious anemia (3 4 6 8 9 10 11 12 15 16 17 18 19 23 24 25 26 27) However other investigators have not studied quantitatively how pernicious anemia patients absorb vitamin B_{12} from the intestine without the mediation of intrinsic factor (IF) nor have they studied the absorption process in cases of pernicious anemia with an acquired resistance to IF preparations or in other types of B_{12} deficiency Furthermore most earlier clinical studies have been made with lower B_{12} doses which cannot be expected to give a safe long term effect in all cases In the present report an account is given of the quantitative *experimental studies* presented in part in earlier papers (3 4) which confirmed that absorption of vitamin B_{12} takes place in two ways

1 Absorption through the mediation of IF

Quantitative absorption studies employing the radioactive isotope technique have shown that the absorption of a certain quantity of B_{12} administered together with an IF preparation increases when the IF dose is increased but only up to a certain maximum (1 4) When the dose of B_{12} given in combination with an optimum amount of IF is varied within the range of 1–100 μg normally IF sensitive patients with pernicious anemia absorb on an average a maximum of 0 5–2 2 μg of the given dose or about the same quantity as the uptake in healthy subjects producing their own IF (3 4 and present Patients with partial resistance to IF p absorb less of the B_{12} dose but

cases be induced to absorb the same quantity of B_{12} if the IF dose is greatly increased (1)

2 *Absorption without mediation of IF*

The studies presented in this paper which were briefly reported earlier demonstrate that *the amount of vitamin B_{12} absorbed from the intestine without the mediation of IF corresponds to approx 1 2 % of the dose administered within a very wide dose range* (In a previous report (4) the uptake was calculated to be approx 1 5 % The new average figure 1 2 % is derived from a more comprehensive material and based on statistical analysis)

Thus by increasing the B_{12} dose sufficiently it is possible to increase the uptake to any desired level since a fairly constant ratio between dose and uptake has been demonstrated throughout the entire dose range from 1 μg to 100 000 μg (100 mg!) The mechanism is not known but the demonstrated relationship between dose and uptake indicates the possibility of a passive diffusion process for which we have used the term direct uptake to differentiate from uptake mediated by IF

It is particularly noteworthy that the direct uptake was found on an average to be of the same magnitude irrespective of whether the patient had completely normal absorption was suffering from anemia with or without resistance to IF preparations or had some other form of disturbed absorption due to idiopathic malabsorption ileitis total gastrectomy ileoectomy or jejunectomy

Finally a summary is given of long term *clinical trials* in pernicious anemia up to more than 5 years in which oral doses of 500–1000 μg vitamin B_{12} without IF were administered daily A preliminary report has been presented earlier (4) The results have shown that the treatment is definitely safe and provides a fully acceptable alternative to injection therapy (Behepan tablets 1 mg KABI) It is effective both in pernicious anemia and in other forms of B_{12} malabsorption Thus older preparations containing IF together with vitamin B_{12} which are less reliable are in our opinion no longer justified (4 5)

MATERIAL AND METHODS

In the experimental studies the oral B_{12} doses were tagged with B_{12}-Co^{60} or B_{12}-Co^{58} (Merck & Co) In most experiments where a very small fraction of the dose was expected to be absorbed 0 75 μC was given as B_{12}-Co^{58} Otherwise a radioactivity dose of 0 1–0.5 μC was administered

The IF concentrate used was a dry commercial preparation from hog pylorus mucosa with a B_{12}-binding capacity of approx 0 2 μg of vitamin B_{12} per mg (2) Of this material 50 mg exert an optimal IF effect with 1 μg of vitamin B_{12} in ordinary pernicious anemia The IF dose needed for maximal absorption increases up to about 500 mg when the B_{12} dose is increased to 10 μg or more (3)

The dose of tagged B_{12} was mixed with the IF concentrate (if any) in water prior to administration The doses were given on an empty stomach if not otherwise specified

The meal used in some tests was standardized to contain bread butter cheese milk and coffee In these tests the dose was given one half hour after the meal

An intramuscular flush dose of 1000 μg of non radioactive cyanocobalamin was given 2 hours after the dose

After each dose 0–24 h urine collections were made Samples of the urines were evaporated to one tenth of their original volume The radioactivity of 5 ml portions of the concentrates was measured with a Tracerlab detector in an arrangement for spectrometric analysis of Co^{60} or Co peaks

The uptake of vitamin B was calculated according to Callender and Evans (7) from the 24 hour urinary radioactivity excretion after conversion of radioactivity to μg of vitamin B_{12} and multiplication by 3 The value thus obtained is not exact since it is based on measurement of excretion not absorption However as discussed in this and earlier reports (3 4) this method gives an approximate value of the amount of B_{12} absorbed which is sufficiently reliable for the validity of the practical conclusions drawn

The diagnoses of the different B deficiency states included in the clinical study were established in each case by the use of a battery of examinations including ordinary hematological tests bone marrow aspiration determination of serum B analysis of gastric juice Schilling tests (with few exceptions) and other tolerance and balance tests of gastrointestinal function The serum B determinations were made with the Euglena gracilis technique by Dr A Killander Institute of Medical Chemistry University of Uppsala Sweden

EXPERIMENTAL STUDIES

Before clinical studies were started extensive experiments were carried out to study the absorption of radioactive vitamin B given orally with and without IF at varying doses and in various clinical conditions

Vitamin B_{12} without IF in pernicious anemia

Tests were carried out with doses varying from 1 μg to 100 000 μg Fig 1 shows the calculated uptake in a double logarithmic diagram For each dose level mean values of calculated uptake and standard deviation are plotted and the number of patients tested is specified

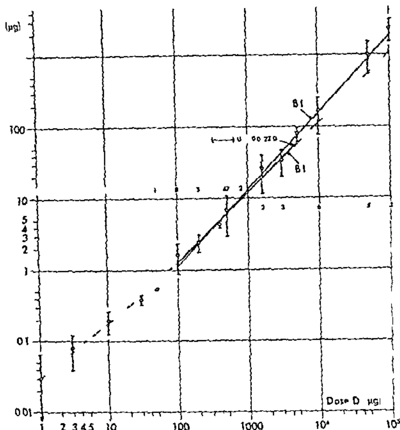


Fig 1 Calculated uptake of vitamin B_{12}, given orally without intrinsic factor to pernicious anemia patients. Mean, standard deviation and number of subjects are specified for every dose level. BI is calculated regression line for interval 100–100 000 µg. *BI* is calculated regression line for interval 100–5000 µg, coincides closely with $U = 0.01$[illegible] D, i.e. uptake = 1.2[illegible] of dose.

BI is regression line for the interval 100–100 000 µg. BI for the limited interval 100–5000 µg.

BI was calculated separately for two reasons:

1. this range has a greater interest for clinical practice.
2. at doses exceeding 5000 µg a significant urinary excretion is obtained also without flush; for this and other reasons (see below) the uptake as calculated may be slightly too high in this upper region.

Equation for regression line BI (100–100 000 µg)

$U = 0.00798\ D$ [illegible]

Equation for regression line BI (100–5000 µg)

$U = 0.0146\ D$ [illegible]

It is interesting to note that the equation for BI within its interval coincides very closely with the simple equation U [illegible] D, i.e. the calculated uptake equals 1.2[illegible] of the dose.

Consequently the average uptake of vitamin B_{12} given orally without IF in pernicious anemia is about 1.2 of the dose within a very wide dose range.

The regression lines do not comprise the range below 100 µg since at low doses the average relative uptake is higher as evident from Fig 1. The natural explanation is the presence of some IF activity remaining in some patients which will influence the total average uptake at low doses.

Vitamin B_{12} without IF in subjects with normal absorption

Tests were carried out with doses from 1 to 800 µg. Fig 2, curve BII shows the calculated uptake. Mean values, standard deviations and number of subjects tested are specified as in Fig 1.

The curve has been drawn by manual fitting. No regression line has been calculated since it cannot be expected to be a straight line and the material is not comprehensive enough to warrant, with any certainty, the calculation of a curve with an equation of a higher degree.

Regression line BI (see Fig 1) is included for comparison.

The result shows that the average absorption at doses from 1 to 100 µg increases slowly from approx 0.5 to 2 µg. As a consequence of the presence of IF in the subjects' gastric juice this absorption is far above the absorption found in pernicious anemia (Fig 1). At higher dosage the absorption curve rises more rapidly as the direct

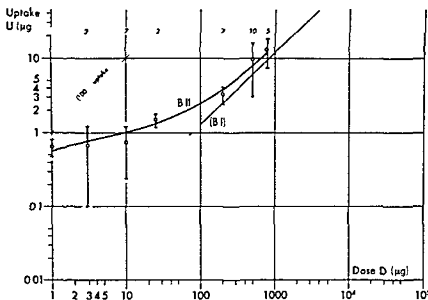


Fig 2 Calculated uptake of vitamin B_{12} given orally without intrinsic factor to test subjects with normal B_{12} absorption *Mean standard deviation and* number of subjects are specified for every dose level Curve BII is drawn by manual fitting. Regression line *BI* (Fig 1) is included for comparison

proportional uptake dominates over the IF mediated absorption The difference between healthy subjects and pernicious anemia patients becomes smaller as the dose is increased showing that the IF mediated absorption is limited and of little importance for the total uptake at high B_{12} doses

The B_{12} uptake in normal subjects has also been studied by Gaffney et al (12) Heinrich et al

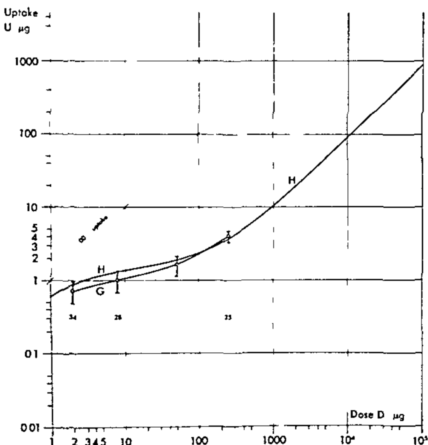


Fig 3 Calculated uptake of vitamin B_{12}, given orally without intrinsic factor to normal subjects, according to other investigators *G* Uptake calculated from urinary excretion values found by Gaffney et al (12) Mean standard deviation and number of subjects are specified for every dose level The curve is drawn by manual fitting. *H* Curve according to an equation for the relationship between dose and uptake suggested by Heinrich et al. (15)

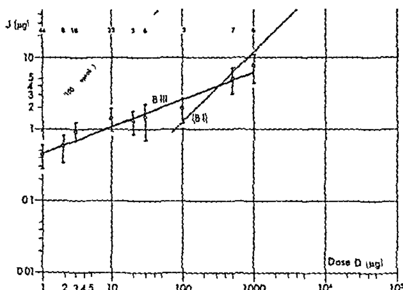


Fig 4 Calculated uptake of vitamin B_{12} given orally with an adequate dose of hog intrinsic factor to pernicious anemia patients Mean standard deviation and number of subjects are specified for every dose level *BIII* is calculated regression line Regression line *BI* (Fig 1) is included for comparison

(15) and Heinrich and Wolfsteller (16) Their results are presented in Fig 3 curves G and H respectively

The average absorption values and standard deviations plotted for curve G were calculated from urinary radioactivity excretion values presented by Gaffney et al in the same way as the uptake was calculated in our own studies

Curve H represents an equation suggested by Heinrich et al for the relationship between dose and total uptake in healthy subjects

$$U = \frac{1.5D}{D+1.5} + \left(1 - \frac{1.5}{D+1.5}\right) 0.009\,D$$

This equation was based on values obtained from total body measurements of absorbed radioactivity after oral administration of radioactive B_{12}

It is evident that curves G and H differ very little and they are also in good agreement with our curve BII

Since the B_{12} absorption ought to be about the same in pernicious anemia patients as in healthy subjects when the doses are high (mainly direct absorption independent of IF) one might also expect good agreement between the upper part of curve *H* and our curve BI Above a dose of 1000 µg Heinrich found in healthy subjects 0.9–1 % absorption (total body measurement) while we found in pernicious anemia approx 1.2 % absorption between 100 and 5000 µg (calculated from urinary excretion) The agreement is fairly good considering the very different techniques used

Vitamin B_{12} with IF in pernicious anemia

Tests were carried out with doses from 1 to 1000 µg vitamin B_{12} together with an optimal amount of IF concentrate The subjects were ordinary pernicious anemia patients i.e. were known not to have developed refractoriness to hog IF From earlier studies (1) we know that IF refractory patients absorb much less B_{12} in this test unless the IF dose is increased very considerably

Fig 4 shows the calculated uptake Mean values standard deviations and number of subjects tested are specified as before

Curve BIII is the regression line calculated from the values obtained

Equation for regression line BIII

$$U = 0.455\ D^{[illegible]}$$

Theoretically the curve cannot be a straight line in this type of diagram since it would cross the 100 absorption limit if extended downwards Within the interval however it ought to give an acceptable description of the relationship between dose and uptake

As expected the uptake is within a wide range approximately the same as that obtained by vitamin B_{12} alone in normal subjects (curve BII) At high B_{12} doses and correspondingly high IF doses however the uptake becomes lower than that obtained from B_{12} without IF in pernicious anemia patients or in normal subjects Obviously the direct B_{12} uptake is hampered by IF and other B_{12} binding material in the hog IF concentrate At B_{12} doses from a few hundred µg and up the IF dose was far higher than the amount normally present in the gastric juice of a healthy subject

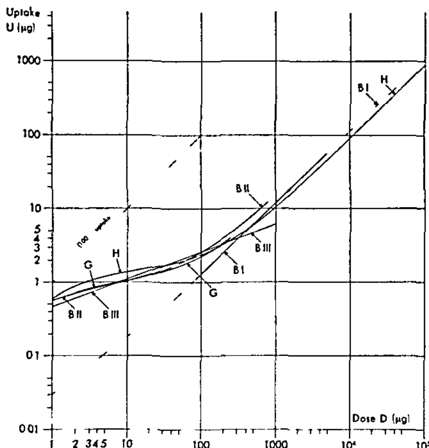


Fig 5 Calculated uptake of vitamin B_{12} curves presented in Figs 1–4 combined for direct comparison B without intrinsic factor in pernicious anemia *BI* Berlin et al., regression line for interval 100–5000 µg. B without intrinsic factor to normal subjects *BII* Berlin et al curve by manual fitting *G* Gaffney et al (17) curve by manual fitting *H* Heinrich et al (15) according to equation B_{12} with hog intrinsic factor in pernicious anemia *BIII* Berlin et al regression line for interval 1–1000 µg

In Fig 5 our curves BI BII and BIII are presented in one diagram together with curve G and curve H from Fig 3 Here a direct comparison of the uptake under various conditions can be made and also a comparison of our results with those of other investigators

Vitamin B_{12} without IF in various conditions individual variations

In addition to the experiments reported above a series of tests has also been made in totally gastrectomized patients and in cases of other types of B malabsorption

Table I shows the calculated uptake in different conditions at the 500 µg dose the level for which the largest number of determinations is available

It is evident that the uptake of a high dose of vitamin B_1 alone is largely the same regardless of the condition of the subject However the average uptake in pernicious anemia patients tends to be slightly lower than in normals probably as a consequence of the lack of IF in their gastric juice A small difference of this magnitude is to be expected At this dose level the IF mediated portion of the total uptake is low compared to the direct uptake (cf curves BI and BII) The direct uptake in pernicious anemia may also be expected to be about the same whether the patients have developed refractoriness to hog IF or not

Fig 6 illustrates the variation at the 500 µg dose level in the entire material Only 3 out of the 74 subjects showed an uptake below 3 µg (the lowest value is 1 8 µg) and 8 had values exceeding 15 µg or 3 % of the dose The majority (70 %) showed an uptake within the interval 4–10 µg or 0 8–2 % of the dose

Effect of flush dose at high B_{12} doses

If the oral B_1 dose is very high the uptake approaches or exceeds 1000 µg i e the size of the intramuscular flush dose For this reason a significant excretion can be expected from high oral B_{1} doses also without flush

A series of tests was carried out with and without flush when B_1 doses of 500 µg and more

Table I Calculated uptake of vitamin B_{12} (µg) in healthy subjects and in various pathological states after an oral dose of 500 µg of radioactive B_{12} on an empty stomach

In brackets are given mean values after the omission of extreme values (< 3µg and > 15 µg)

Normal (10 cases)	Pernicious anemia, IF sensitive (37 cases)			Pernicious anemia IF refractory (9 cases)	Malabsorption (13 cases)
3.5	2.6	5.6	7.2	1.8	3.3
5.4	2.7	5.6	7.4	3.5	4.5
5.6	3.6	5.7	8.3	4.1	4.5
6.3	3.8	5.9	8.4	4.2	4.8
7.5	3.9	5.9	8.4	5.6	5.1
7.7	4.4	5.9	9.0	6.2	6.3
8.7	4.8	5.9	9.6	6.3	6.8
11.7	4.8	6.3	13.4	9.6	7.7
14.0	5.0	6.5	13.8	14.0	12.5
16.3	5.0	6.6	16.5	Mean 6.1 µg	16.1
Mean 9.7 µg	5.0	6.8	21.9	(6.7 µg)	16.4
(7.8 µg)	5.1	6.8			22.1
	5.4	6.8			29.4
	Mean 7.0 µg				Mean 10.7 µg
	(6.6 µg)				(6.2 µg)

Total gastrectomy (3 cases)	Jejunectomy (1 case)	Ileitis (1 case)
5.5	9.3 µg	17.6 µg
7.4		
9.0		
Mean 7.3 µg		

Total material (74 cases) Mean 8.1 µg (6.8 µg)

were given The results are shown in Table II Doses of 500 µg still need the intramuscular flush dose for a significant urinary excretion whereas at 10 000 µg the excretion without flush approaches the excretion after flush After B_{12} doses of 50 000–100 000 µg the oral B_{12} dose apparently acts as a full flush dose in itself since the radioactivity excreted is about the same with and without flush

Since after very high oral B_{12} doses the relationship between oral absorption and 24-hour urinary excretion has not been determined we do not know if the factor 3 used by us and found by Callender and Evans (7) at lower doses is reliable Nevertheless the absorption must be at least as high as or higher than the amount actually found in urine during 48 hours (cf ?) Table II shows that the amount absorbed is of a magnitude of about 1% of the dose or more also when the highest doses are given Even if the ratio absorption to 24-hour excretion may thus differ from 3 at high doses (cf 15 and Fig 5 curve H) this uncertainty has no decisive influence on the conclusions drawn from the studies presented here

Influence of food intake on B_{12} absorption

Finally a study was made to find out if the B_{12} absorption of a dose given after a meal was different from the absorption of a dose taken on an empty stomach Table III gives the result of measurements in ten test subjects at a dose of 500 µg

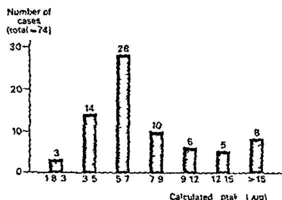


Fig 6 Calculated uptake of vitamin B_{12} following an oral dose of 500 µg radioactive B_{12} taken on an empty stomach Distribution of the entire material (74 subjects) comprising normal person and patients with pernicious anemia or other gastrointestinal absorption disturbances

Table II *Effect of intramuscular flush dose on urinary B_{12} excretion after high oral doses of vitamin B_{12}*

Oral dose of radioactive B_{12} (μg)	Per cent of radioactivity recovered in urine: With flush 0–24 h	With flush 0–48 h	Without flush 0–24 h	Without flush 0–48 h
500	0 55	0 65	0 03	0 05
500	0 48	0 57	0 01	0 02
500	0 43	0 52	0 02	—
500	0 60	0 75	0 09	0 10
500	0 92	1 38	0 05	0 11
500	0 30	0 42	0 00	0 02
500	0 89	1 [illegible]9	0 01	0 02
500	0 70	1 18	0 05	0 10
5 000	0 55	—	0 03	0 05
5 000	0 71	1 10	0 24	0 27
5 000	0 43	0 5[illegible]	0 06	0 19
10 000	0 67	0 85	0 12	0 25
10 000	0 79	0 98	0 69	0 80
10 000	0 97	1 08	0 51	0 54
25 000	0 28	0 57	0 18	0 19
50 000	0 35	0 6	0 94	1 08
50 000	*0 69*	*0 91*	*0 75*	*0 83*
50 000	0 65	1 00	0 57	0 67
50 000	—	—	0 53	0 77
100 000	0 48	0 70	0 41	0 58
100 000	0 97	1 16	0 88	1 09

[a] The intramuscular flush dose of 1000 μg of non radioactive B_{12} was given 2 h after the oral dose and repeated 24 h after the oral dose

It is obvious that the uptake is moderately higher when the dose is taken on an empty stomach The dose should therefore preferably be taken before or between meals although this does not seem to be a critical point

CLINICAL STUDIES

Several authors have attempted to determine the daily requirement of vitamin B_{12} in order to obtain a criterion for establishing the necessary daily uptake in the treatment of pernicious anemia

Table III *Calculated uptake (μg) of vitamin B_{12} on an empty stomach and after a standard breakfast*

Oral dose 500 μg radioactive B_{12} Mean values and range for ten patients with pernicious anemia

	Fasting	After meal
Calculated uptake (μg)	7 8 (4 8–13 4)	4 8 (1 8–7 5)

Table IV *Observation period for long term oral treatment of pernicious anemia with 500–1000 μg of vitamin B_{12} (64 cases)*

All six patients treated for three years or less and a few of the others, died during the observation period for reasons not connected with the disease studied

Time (mo)	No of cases	Time (mo)	No of cases
70	2	35–39	3
65–69	4	30–34	—
60–64	11	25–29	1
55–59	17	20–24	1
50–54	8	15–19	—
45–49	5	10–14	1
40–44	11		

It may be assumed that about 2 μg constitutes the daily turnover rate and an amount sufficient to prevent relapse in pernicious anemia (14 20 21) Even though other studies (13 20 22) have indicated a higher requirement a quantity of about 2 μg corresponds fairly well to practical experience

As demonstrated above an oral dose of 500–1000 μg of vitamin B_{12} gives on an average an absorption of 6–12 μg Even when individual variations are taken into account a daily dose of this magnitude can be expected to give a safe maintenance effect

Altogether the *clinical material* comprises 64 established B_{12} deficiency cases from three Swedish medical centers (Eskilstuna Falkoping and Linkoping) all examined and supervised personally by one of the authors (R. Berlin or G Brante) The patients were started on 500 or 1000 μg of B_{12} daily during the last three years of the study 1000 μg daily were given throughout Sixty-one of the patients have been under treatment more than 36 months 44 more than 48 months and 17 during 60 months or more A more detailed review of the observation period of the entire material is shown in Table IV The clinical diagnoses are given in Table V

The *clinical results* which are presented in Table VI Fig 7 and Fig 8 are more positive throughout to our knowledge than any results reported earlier in oral treatment of pernicious anemia It should be noted that in this study a strict evaluation of the effect was made including regular determination of serum B_{12} for all patients The patients were encouraged to adhere strictly to

V *Case material in long term clinical trials with 500 µg or 1000 µg of vitamin B_{12} mistered daily in tablet form*

	Pernicious anemia		Malabsorption			
	Ordinary	Resistant[a]	After ileal resections	Cause not established	Total gastrectomy	Total
o of cases	18	37	4	4	1	64

Demonstrated resistance of various degrees to preparations containing hog intrinsic factor plus vitamin B_{12} or li er eria falling values for Hb and red blood cells and/or low values for B_{12} in blood serum during treatment with hog IF subnormal response in the Schilling test with B_{12} + hog intrinsic factor

able VI *Results of oral long term therapy with 00–1000 µg of vitamin B_{12} daily in tablet form*

ixty four cases of pernicious anemia and other types of vi in B_{12} deficiency

b and RBC	Satisfactory values 55 cases	Subnormal values 9 cases[a]	Total 64 cases
B_{12}	Normal 64 cases	Subnormal 0 cases	

One gastric carcinoma one chronic pyelonephritis post operative abscess of left th gh one metastasing urinary blad carcinoma one chronic uremia one gastric carcinoma menorrhagia + iron deficiency anemia one ileitis with ngs one iron deficiency after total gastrectomy one idiopathic malabsorpt on

the medication and were interviewed at each visit for checking of their medication habits They were also instructed to dis ontinue the treatment for three days before the blood sample was to be taken in order to avoid temporary peaks in the serum B_{12} level

The therapy was discontinued for six patients in order to study the time needed for reappearance of low serum B_{12} values The patients were kept under strict clinical control Table VII shows that the serum B_{12} values stayed well within normal limits in all the patients for about 4 months Three patients reached the lower normal limit of 150 pg/ml after about 5 months and one patient after 7

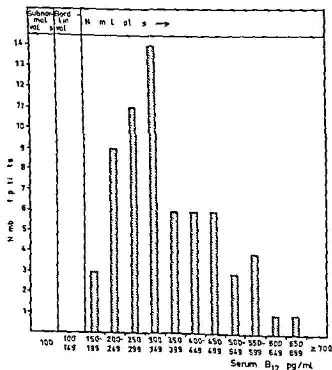


Fig 7 Results of oral long term therapy with 500–1000 µg vitamin B_{12} daily in tablet form mean values of all serum B_{12} determinations made from the second month of treatment Sixty four cases of pernic ous anemia and other types of vitamin B_{12} deficiency

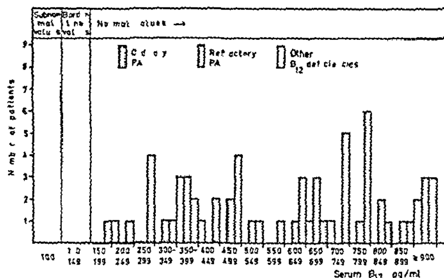


Fig 8 Results of oral long term therapy with 500–1000 μg vitamin B_{12} daily in tablet form serum B_{12} values at the end of the follow up period The material (64 cases) is divided into groups according to type of B deficiency

months Two patients still had values within the normal range after 8 ¹/ and 7 months respectively In a further case (B_1 deficiency after total gastrectomy) all B_1 therapy was interrupted by inadvertence after 44 months of oral treatment with 500 μg of B_1 daily After 1 ¹/ years his serum B_1 value had decreased from 570 to 350 pg ml In no case did the values for hemoglobin red cells serum iron iron binding capacity serum lactic acid dehydrogenase or serum glutamic acid oxalacetic acid transaminase show any notable changes during the observation periods The results indicate that *oral treatment with 500 μg of B_{12} daily had also replenished the B_{12} depots*

Fig 7 shows the mean values of all serum B_1 determinations made for each case after the initial two months of therapy Fig 8 presents the last serum B_1 values determined in the follow up study In this figure the material is divided according to type of B_1 deficiency Of all serum B_1 determinations made after 2 months of treatment or more comprising 562 values only 40 values were below 150 pg/ml and 6 values below 100 pg/ml Generally these low values were obtained during the early months of treatment of patients in definite relapse Repeated low values on several consecutive occasions were found only in exceptional cases where 500 μg daily was given they could easily be corrected by increasing the dose to 1000 μg daily Thus the long term treatment with high doses of vitamin B_1 alone has resulted in serum B_1 levels well within the normal range in all cases

It should also be noted that no negative reactions were recorded that could be attributed to the medication No observations were made of

Table VII *Serum B_1 values after discontinuance of oral B_1 therapy 500 μg daily (after the time for last value reported the treatment was resumed)*

Patient and type of B_{12} malabsorption	Duration of treatment when discontinued (mo)	Serum B_1 (pg ml) time after discontinuance: Days 3	Days 10	Months 1		3	4	5	6	7	8
1 Ordinary PA[a]	19	440	00	?05	190		190		150		130
2 Ileal resection	.3	515	460		210	210		110	140		
3 IF refractory	33	315	470		170	210		145	170		
4 Idiopathic malabsorption	33	390	400		55	290		?10		135	05
5 Ileal resection	33	?65	300		. 0		230		.35		
6 IF refractory PA	33	570	510		?35	265		260		2 0	

[a] PA = pernicious anemia

neurological disturbances indicating development of subacute combined degeneration of the cord Absent ankle tendon reflexes recorded in a few cases were connected with concomitant diseases such as diabetes advanced arteriosclerosis or a history of sciatica Absence of vibratory sensibility in the feet was reported in one case an old woman with senile dementia

Many patients spontaneously expressed satisfaction with the discontinuance of injections At a special interview some patients declared that they did not care whether they received injections or tablets No patients had any objection to oral treatment

Since the uptake is proportional to the dose it has been possible to supply the entire daily requirement by *one* small tablet daily Only very few patients tended to neglect the medication In all but one a mentally deficient patient negligence could be corrected by information

With regard to the daily standard dose—500 μg or 1000 μg—the higher dose was chosen Although it was found both theoretically and through experience that 500 μg per day gave satisfactory results the higher dose offers better protection in the event of temporary negligence in medication Even at the higher dose the treatment is simple and inexpensive and no report has been received of any side reactions or other unfavourable effects from long term therapy

The investigation has also demonstrated that it is possible to give oral treatment with high doses of vitamin B_{12} not only as maintenance therapy but also as initial treatment during relapse without any untoward effects For investigational reasons the maintenance dose has been used also initially throughout this study In routine use we recommend however initial oral treatment of 2000 μg twice daily during the first month or injection therapy in order to fill the body stores as rapidly as possible

Our clinical experience has provided complete confirmation of the experimental results It is now definitely established that the treatment of pernicious anemia with high oral doses of vitamin B_{12} without IF is a fully satisfactory alternative to injection therapy not only in cases of ordinary pernicious anemia but also in those showing IF refractoriness and in other cases of B_{12} malabsorption

This treatment although the cost of the tablets is somewhat higher than the cost of injectable B_{12} is economically quite reasonable because it saves personnel and allows less frequent visits to the doctor The tablets were introduced in Sweden in 1964 and are now in widespread use No negative experiences have been reported in connection with their practical routine use

ADDENDUM

In a review of his experimental work on oral B_{12} without IF Heinrich (Ergebn inn Med Kinderheilk. 25 1 1967) discusses the suitable daily maintenance dose and finds 300 μg daily to be adequate

Statistically this is correct because this dose results in an absorbed and retained average quantity of approx 3 μg or slightly more than the normal daily turnover However considering the variations in absorption, a wider safety margin seems to be appropriate in order to provide a safe standard therapy Heinrich's figures as well as ours show some absorption values far below average and in our experience low values are found rather constantly in certain individuals Heinrich refers to clinical trials published by others indicating that 300 μg daily might be sufficient Our more comprehensive material followed up for a much longer period of time showed that 500 μg daily although in general adequate may give borderline serum B_{12} values in a few cases In these patients normal values were rapidly restored by 1000 μg daily

Considering that some patients may have a higher B_{12} requirement or a lower uptake than average and further that (*a*) the absorption is slightly lower if the dose is taken with a meal (*b*) a reasonable allowance for occasional negligence should be taken into account in this type of self medication (*c*) a dose of 1000 μg daily has not caused any untoward reactions during 5 years study and (*d*) the difference has little economic significance we have preferred to recommend 1000 μg as daily maintenance dose

Heinrich also discusses much higher oral doses at correspondingly longer intervals We have seriously considered such alternatives since it is no doubt possible to work out other dosage regimens which are equally effective In our opinion however it is necessary that an important selfmedication like this can be included in a simple daily routine The possible cost reduction gained by higher doses at longer intervals would most probably not be large enough to justify the increased risk of the patient's forgetting his tablet and the more serious consequences if a dose is neglected

REFERENCES

1 Berlin R Berlin H *Brante* G & Sjoberg S G Refractoriness to intrinsic factor B_{12} preparations abolished by massive doses of intrinsic factor Acta med scand 16? 317 1958

— Studies on intrinsic factor and pernicious anemia I Oral uptake of vitamin B_{12} in pernicious anemia with increasing doses of an intrinsic factor concentrate Scand J clin Lab Invest 10 ?78 1958

3 — The absorption of IF bound and free B_{12} in various clinical conditions Paper read at the 2nd European Symposium on vitamin B and intrinsic factor Hamburg 1961
4 Berlin, H., Berlin R. & Brante G Peroral behandling av pernicios anemi med hoga doser vitamin B utan intrinsic factor Lak Tidn 6? 773 1965
5 — Crude or refined intrinsic factor in preparations for the oral treatment of pernicious anaemia Scand J Haemat 3 236 1966
6 Brody F A Estren S & Wasserman L R Treatment of pernicious anemia by oral administration of vitamin B without added intrinsic factor New Engl J Med 260 361 1959
7 Callender S T & Evans J R The urinary excretion of labelled vitamin B_{12} Clin Sci 14 295 1955
8 Chalmers J N M & Hall Z M Treatment of pernicious anaemia with oral vitamin B without known source of intrinsic factor Brit. med J 1 1179 1954
9 Chalmers J N M & Shinton N K Absorption of orally administered vitamin B_{12} in pernicious anemia Lancet 2 1 98 1958
10 Conley C L & Krevans J R New developments in the diagnosis and treatment of pernicious anemia Ann intern Med 43 758 1955
11 Doscherholmen A & Hagen P S A dual mechanism of vitamin B plasma absorption J clin Invest 36 1551 1957
12 Gaffney G W., Watkin D M & Chow B F Vitamin B absorption relationship between oral administration and urinary excretion of cobalt[60] labeled cyanocobalamin following a parenteral dose J Lab clin Med 53 5?5 1959
13 Grasbeck R Calculations on vitamin B turnover in man with a note on the maintenance treatment in pernicious anemia and the radiation dose received by patients ingesting radiovitamin B Scand J clin Lab Invest 11 50 1959
14 Heinrich H C & Pfau A A Der Einsatz von Gesamtkorper Radioaktivitats Detektoren mit flussigen organischen Szintillatoren in der klinischen Radioisotopen Diagnostik und Forschung Atomkernenerg e 6 463 1961
15 Heinrich H C Gabbe E E., Whang, D H & Wolfsteller E Eine fur die Berechnung der intestinalen Vitamin B Resorption beim Menschen sowohl im physiologischen Intrinsic Factor abhangigen als auch im unphysiologischen hohen diffusionsbedingten Dosisbereich allgemein gultige Formel Z Naturforsch 0 b 1067 1965
16 Heinrich H C & Wolfsteller E Hochdosierte orale Vitamin B Therapie Med Klin 61 756 1966
17 McIntyre P A., Hahn R., Masters J M & Krevans J R Treatment of pernicious anemia with orally administered cyanocobalamin (vitamin B) Arch intern Med 106 280 1960
18 Meyer L M Sawitsky A Cohen B S Krim M & Fadem R Oral treatment of pernicious anemia with vitamin B Amer J med Sci 2.0 604 1950
19 Reisner E H., Weiner L., Schittene M T & Henck E A Oral treatment of pernicious anemia with vitamin B without intrinsic factor New Engl J Med. .53 50? 1955
20 Reizenstein P G Vitamin B_{12} metabolism Acta med scand Suppl 347 1959
21 — Excretion of non labeled vitamin B_{12} in man Acta med scand 165 313 1959
22 — Body distribution turnover rate and radiation dose after the parenteral administration of radiovitamin B_{12} Acta med scand 165 467 1959
23 Ross G I M., Mollin D L., Cox E V & Ungley C C Hematologic responses and concentration of vitamin B_{12} in serum and urine following oral administration of vitamin B_{12} without intrinsic factor Blood 9 473 1954
24 Shinton N K Oral treatment of pernicious anemia with vitamin B_{12} peptide Brit med J 1 1579 1961
25 Spies T D Stone R E Lopez G G Milanes F Toca R L & Aramburu T Vitamin B_{12} by mouth in pernicious and nutritional macrocytic anaemia and sprue Lancet 2 454 1949
26 Unglaub W G Rosenthal H L & Goldsmith G A Studies of vitamin B in serum and urine followin oral and parenteral administration J Lab clin Med 43 143 1954
27 Waife S O Jansen C J Jr Crabtree R E Grinnan E L & Fouts P J Oral vitamin B without intrinsic factor in the treatment of pernicious anemia Ann intern Med 58 810 1963

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TREATMENT OF ANGINA PECTORIS WITH BETA RECEPTOR BLOCKADF MODE OF ACTION

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Abstract In a double blind cross-over study the effect of the dextro isomer and the racemate of a beta receptor blocking agent (H56/28®) was compared with that of a placebo in 13 patients with angina pectoris The dextro isomer had no effect on attack rate The inhibitive effect of racemate on angina pectoris was highly significant statistically The decrease in the number of angina pectoris attacks was of such magnitude that it was considered also of clinical significance in the majority of the patients The difference in effect of the dextro isomer and of the racemate indicates that the inhibitive effect on angina pectoris is due to the laevo isomer The main beta receptor blocking activity resides in the laevo isomer and thus the beneficial effect on angina pectoris seems to be due to beta receptor blocking properties No definitely serious side-effects were observed

Several investigations published during the past two years seem to indicate that beta receptor blocking agents have a positive effect on angina pectoris as recently reviewed by Epstein and Braumwald (4) Such investigations are complicated by many factors even in controlled double blind trials as recently discussed (2) The so-called run in effect can interfere with a subsequent double blind trial by causing a continuing decrease of angina pectoris attacks during this trial (5) or by diminishing the symptoms to such an extent that the following double blind trial becomes difficult to evaluate (2) Such a run in effect on angina pectoris has been demonstrated also with agents that are ineffective (3) It is also important to control other factors such as a possible carry-over effect between trial periods as discussed by Rabkin et al (6) Moreover it seems necessary to perform double blind trials with the patients knowing that a placebo will be introduced during unknown periods of the trial otherwise there appears to be a risk of a biased recording of attacks as pointed out previously (2)

Not until these technical problems are controlled is it possible to approach the more central question of the mode of action of beta receptor blockade in angina pectoris It is thus not known whether the effect is actually due to beta receptor blockade or to other causes e g local anesthetic effects This question has arisen because it seems that much higher doses are required in order to produce an effect on angina pectoris than those causing beta receptor blockade Information on this matter seems to be of interest not only from the therapeutic point of view but also because it may contribute to our knowledge of the nature of angina pectoris When planning the present study the difficulties referred to were avoided by selecting only patients with frequent attacks of angina pectoris Furthermore the run in effect on the double blind trial was minimized by prolonging it to over four months and by administering the same number and size of tablets during the trial and not as in a previous study (2) increasing the number of tablets Carry over effects were compensated for by analysing only late parts of the periods of the double blind trial Finally patients were informed about the introduction of a placebo during unknown periods of the trial The beta receptor blocking agent used was 1 (2 allyl phenoxy)-3 isopropylamino propanol (2)hydrochloride (H 56/28 Aptin® AB Hassle Goteborg)

With this technique it was possible to investigate the effectiveness on angina pectoris of the dextro- and laevo isomers of H 56/28 by testing first the pure dextro-form and then for the same case material the racemate This was of interest

Table 1 *Clinical data and results*

Pat no	Age	Sex	Diagnosis	Dextro trial: No of attacks per 14 d		Racemate trial: No of attacks per 14 d		Subjective impression[b]	B P[c] (mm Hg)
				Placebo	Dextro	Placebo[a]	Racemate		
1	69	♂	Ang. pect. hypertension	5	8	9 (8)	4	+2	0 0
2	76	♀	Ang pect. diabetes mellitus hypertension	10	7	12 (8)	4	+4	−10 7
3	54	♀	Ang. pect. diabetes mellitus, hypertension cholelithiasis, hypercholesterolemia	7	14	8 (8)	7	−1	−25 −3
4	66	♂	Ang. pect	11	5	2 (8)	0	+3	−2 10
5	63	♂	Ang. pect hypertension	18	11	10 (4)	0	+5	0 0
6	66	♀	Ang pect hypercholesterolemia	9	9	10 (9)	7	+1	−10 −10
7	61	♂	Ang pect. hypercholesterolemia	25	18	23 (30)	14	+1	−3 10
8	69	♀	Ang pect	6	10	6 (5)	3	+3	−15 7
9	70	♂	Ang pect. hypercholesterolemia	111	106	84 (90)	55	+3	10 5
10	61	♂	Ang pect	4	19	6 (12)	4	0	0 0
11	51	♂	Ang. pect hypercholesterolemia	52	42	3 (4)	17	0	0 0
12	66	♀	Ang. pect.	26	36	—	—	—	—
13	64	♂	Ang pect. hypercholesterolemia hypertension	5	5	—	—	—	—
			Sum	289	290	173 (186)	115		

[a] Figures within parentheses refer to the placebo period immediately before the racemate trial (cf Material and methods)
[b] General condition during treatment (racemate) and placebo periods as estimated by the patient compared with the preceding weeks unchanged 0 improved +1 much improved +2 worse −1 much worse −2. The table shows the score for *the treatment period minus that for the placebo period*
[c] The figures indicate change in systolic diastolic blood pressure calculated as mean systolic diastolic blood pressure during placebo periods minus that during treatment (racemate) period

for the elucidation of the mode of action since isomers have approximately the same local anesthetic effect but the beta receptor blocking effect is much stronger in the laevo-form (1)

MATERIAL AND METHODS

Fifteen outpatients with angina pectoris were selected for the trial Two of these had to be excluded early in the *investigation because of inability to cooperate The re*maining 13 patients consisted of five women aged 54–76 and eight men aged 51–70 On exertion, all suffered from typically localized anginal chest pains which im*mediately subsided when patient was at rest nitroglycerin* alleviated the pain. Seven patients had previously suffered from myocardial infarction In none of these cases did infarction occur less than three months before the trial. Two patients were *subjected to coronary angiography* showing pronounced pathological lesions When the study was begun all the patients had had not less than four (11 more than 7) attacks of angina pectoris per week, lasting at least two minutes Six *patients were treated* with digitalis, with or without saluretics for previous congestive heart failure four for hypertension, and five for hyperlipemic conditions Two had diabetes mellitus treated with diet and sulphonylurea None had a history of obstructive lung disease or a tendency to mental depression During the trial no changes were made in previous medication including the above mentioned drugs and also long acting nitrite preparations (9 patients) sedatives and anticoagulant therapy All took nitroglycerin but were recommended not to use it prophylactically

That part of the study in which the dextro form of H 56 28 was tested was started in March 1967 with a one week control period followed by a two-week placebo *period Then 25 50 75 and 100 mg of the dextro isomer* were given four times daily each dose for one week *Thereafter* followed two *double blind* periods of placebo or 100 mg of the dextro isomer four times daily in randomized order each for three weeks During this whole period of 13 weeks except for the first control week, *four tablets of identical appearance and taste were given daily*

During weekly checkups patients data-sheets were collected history of the preceding week recorded and physical examination and ECG and other laboratory tests *performed as described previously ( )* with the exception that blood lipids and chest X-ray were not followed in all patients

Immediately after the last double-blind period of the *dextro trial a four week placebo period was introduced* This was during the vacation period (July) Frequency of attacks was recorded during the last two of these four weeks Then followed two double-blind periods of placebo *or 100 mg of the racemate of H 56 28 given four* times daily in randomized order each for three weeks

During this part of the trial patients no 1? and 13 (cf Table I) were unable to take part because of lack of time During this whole period of ten weeks four tablets of identical appearance and taste were again taken daily which means that during 16 weeks prior to the racemate trial the same number of similar tablets was taken daily

During this latter part of the trial the number of attacks was recorded and the history and physical and laboratory status controlled as in the dextro trial

Statistical calculations were made one tailed according to Wilcoxon rank sum test (7)

RESULTS

The results of the dextro trial are shown in Fig 1 and Table I The number of angina pectoris attacks were practically identical when the last two weeks of the placebo and the treatment periods are compared This applied also to nitroglycerin consumption (right half of Fig 1 left part of Table I)

This result seemed to justify regarding the whole of this part of the investigation as a run in period for the racemate trial Therefore in the left half of Fig. 1 the number of attacks and the nitroglycerin consumption per week have been marked off with the weeks in consecutive order irrespective of whether placebo or the dextro isomer was given It is then immediately apparent from the figure that the run in effect was very small

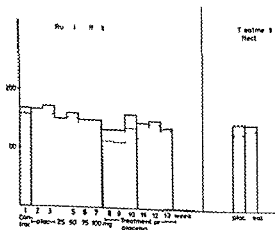


Fig 1 Number of attacks of angina pectoris and number of nitroglycerin tablets taken during different periods of the trial of the dextro isomer of H 56/28 Left part of figure shows effect of time on number of attacks and nitroglycerin consumption right part shows average number of attacks and nitroglycerin tablets taken per week during the two last weeks of the double blind trial with the placebo or the drug, each for a total period of three weeks

Furthermore the average number of angina pectoris attacks during weeks 11 12 and 13 (see Fig 1) was not lower than that during weeks 8 9 and 10 (144 and 142 respectively) showing that any possible run in effect had passed by this time

The results of the racemate trial are given in the right part of Table I All the patients had a smaller number of angina pectoris attacks when taking racemate as compared with the placebo results except for patient no 11 This difference is highly significant statistically ($p = 0\ 006$) The nitroglycerin consumption was closely related with the number of attacks and is not presented Eight out of the 11 patients remaining in this part of the trial had a positive score when they assessed their own condition while two rated themselves as 0 and one had a negative score Whether the changes in blood pressure are of significance seems uncertain

Side effects

The following observations may possibly be pertinent to a discussion of side-effects although they may equally well be unrelated to drug administration For patient no 6 the serum glutaminoxalacetic acid transaminase values were 35 and 45 units for the last two weeks before the introduction of racemate 60 units during its introduction and 18 units immediately after Initially patient no 3 had normal alkaline phosphatase values which rose during placebo administration to 17 and 14 units when on racemate her value was 12 This patient had cholelithiasis Her other liver tests were normal Patient no 5 developed gynecomastia bilaterally during the trial No changes in this condition were observed whether the patient received placebo tablets or the drug No other subjective or objective signs of any possible side-effects were observed

DISCUSSION

The first part of the study showed that compared with the placebo the dextro isomer of H 56/28 had no effect on the number of angina pectoris attacks This result made it possible to consider the whole first part of this investigation as a run in period for the latter part where the racemate of H 56/28 was tested It was also demonstrated that the attack rate was constant indicating that the run in effect of the utilized procedures had passed This fact facilitates the interpretation of the results of the racemate trial which followed

There was however a decrease of approximately 100 attacks in the placebo period immediately before the racemate trial (173 (186) see

sum of attacks at bottom of Table I) as compared with preceding periods (289 290) It should be noted that in addition to the missing attacks of the two patients who did not participate in this part of the trial this was almost entirely due to patient no 11 who spontaneously stated that his angina pectoris was much improved by his vacation Consequently it is most probable that this decrease in the total number of attacks is not caused by a continuing run in effect

In a previous study (2) it was found that the number of angina pectoris attacks diminished markedly when the dose of the racemate was increased *during the run in period* In view of the information on the true magnitude of the run in effect in the present work it seems probable that this marked decrease was caused to a significant degree by the drug even though the mode of tablet administration was different in the two investigations The fact that in the study mentioned (2) frequency of attack did not again increase during the subsequent placebo periods to approximately the level of the control values might well be due to either a carry-over effect and/or the physical training of the patients (cf 8) which was made possible during the run in period because of fewer angina pectoris attacks

When run in effects and carry-over effects were *excluded and a sufficient number of attacks remained* to allow comparisons betwen periods the effect of the racemate in preventing angina pectoris attacks was found to be highly significant statistically

The clinical significance of the effect of the drug evidently varied in different patients Most of the patients were helped by the treatment This is clearly seen in the number of attacks in patients no 2, 5 7 and 9 The condition of other patients also improved even though this is not so evident in the number of attacks Patients no 1 4 and 8 were able to increase their physical activity and did so up to the limit where angina pectoris again occurred This is most dramatically exemplified by patient no 1 who when the trial started was unable to leave his house because of angina pectoris but when on racemate he climbed the roof of his house in order to repair it and while doing this again had angina pectoris The rest of the patients however did not experience for certain any beneficial effect of the treatment (no 3 6 10 and 11) Patient no 11 even had an increased number of attacks of angina pectoris when treated with racemate According to the patient this was probably due however to personal problems which caused anxiety and sleeplessness suddenly arising during the period of the racemate treatment

It then seems possible to conclude that the racemate of H 56/28 has a definite statistical effect on the frequency of angina pectoris attacks The clinical significance of this decrease in the number of attacks varies in different patients but no doubt most patients are helped by this treatment What characterizes these patients who experience a beneficial effect is not apparent at present The dextro isomer of H 56/28 has only a local anesthetic effect and very weak beta receptor blocking activity (1) It is not effective in the treatment of angina pectoris Therefore the local anesthetic effect of the racemate probably does not account for the prevention of angina pectoris attacks It appears probable that this effect is exerted by the beta receptor blocking activity of the laevo isomer of the racemate

REFERENCES

1 Ablad B., Brogård M L & Ek L. Acta pharmacol (Kbh) Suppl 2 9 1967
2 Bjorntorp P Acta med scand 18. 785 1967
3 Cole S L., Kaye M K. & Griffith G C JAMA 168 275 1958
4 Epstein S E & Braumwald E New Engl J Med 275 1106 1966
5 Jacobsson K A Koch G., Levander Lindgren, M & Michaelson G Acta med scand 180 1 9 1966
6 Rabkin R. Stables D P., Levin, N W & Suzman N M Amer J Cardiol 18 370 1966
7 Siegel S Nonparametic statistics p 75 McGraw Hill New York 1956
8 Varnauskas E Bergman H Houk P & Björntorp P Lancet 2 8 1966

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FREE FATTY ACIDS GLYCEROL AND ALVEOLAR ACETONE IN OBESE WOMEN DURING PHENFORMIN TREATMENT

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Abst act One patient, acting as her own control was studied for twenty months She lost weight when on phenformin and gained weight when this was discon tinued A group of moderately obese subjects lost 1 3 kg in four weeks on phenformin as against 0 8 kg on placebo In a second group with resistant obesity no weight changes were observed. The alveolar concentration of acetone and the plasma concentration of FFA did not change significantly during the observation periods whereas the plasma concentration of glycerol was significantly reduced during the phenformin period

Concomitant with the increasing prevalence of administrating phenformin hydrochloride to pa tients with diabetes it has been observed that phenformin has effects over and above those on carbohydrate metabolism During recent years this drug has been particularly recommended to obese diabetics This indicates that phenformin could be of special importance for fat metabolism (1 3 7 14) Very few studies of phenformin have been made on non-diabetic patients (12) Pedersen (11) treated four non-diabetic obese subjects with metformin which is closely related to phenformin and has a similar action He observed that during caloric restriction the weight loss was greater with metformin than without it

It was incidently observed that a non-diabetic patient on caloric restriction lost *weight* considerably faster on phenformin In a subsequent preliminary trial three out of five women showed a marked weight loss on phenformin whereas other forms of treatment had failed to produce this result It was therefore of interest to ascertain whether the weight reducing effect of phenformin could be observed in a control clinical study In view of the interaction between the carbohydrate metabolism and the fat metabolism free fatty acids (FFA) and glycerol in plasma were deter mined and the acetone in the alveolar air was estimated as an indication of the levels of the arterial ketone bodies (13)

MATERIAL

One overweight woman (weight 9 kg height 166 cm) a nurse born 1918 without any family history of diabetes and normal glucose tolerance test was studied for twenty months with regard to weight loss on a caloric intake of 1500 kcal/day with and without phenformin administra tion and for one period on a caloric intake of 500 kcal day She became obese during the last ten years and was meticulously attentive to caloric intake during the study

Two series of subjects were stud ed in a controlled clinical trial The first consisted of 13 female subjects who considered themsel es overweight and wanted to reduce According to Natvig s standard four of them were not overweight The patients were observed e ery fortnight when they were weighed and their alveolar acetone concentration determined The fasting, plasma concentrations of FFA and glycerol were determined on venous blood after both a fortn ght on phenformin and a fortnight on placebo Each subject was one month on placebo and one month on phenformin The phenformin dose was 50 mg in enterocoated capsules twice a day The drugs were allocated according to the usual double blind te hnique

The second series consisted of 18 women and three men with long-standing obesity which had been treated for a considerable time at the Department of Medicine At the time that the study was in progress they were all living at home and attending the outpatient department Farher they had taken part in a control clinical study to test an appetite depressant These patients were regarded as representing a particularly therapy res stant group They were seen once a month

Weight and concentration of acetone in the alveolar air were followed as in the previous group In this series the plasma concentrations of FFA and glycerol were de termined after one month either on placebo or on phen formin. Not all of the subjects were fasting, as some had taken coffee or tea and one or two p eces of bread and butter about two hours before the venous blood samples were drawn

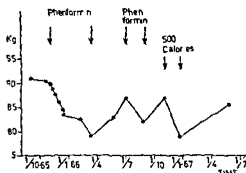


Fig 1 The weight curve of the nurse born in 1918

The patients were only given the drugs either phen formin or placebo no dietary instructions were given for either series

METHODS

The acetone concentration in the alveolar air was de termined by gas-chromatography according to Rooth and Östenson (13)

Plasma FFA were analyzed by a colorimetric method described by Laurell and Tibbling (9) and plasma gly cerol by an enzymatic fluorometric micro-method ac cording to Laurell and Tibbling (8)

RESULTS

The weight changes in the nurse studied for twenty months are shown in Fig 1 During the first twelve months she was on the same caloric intake When taking phenformin she rapidly lost weight whereas when the drug was not adminis tered she gained weight However she twice ceased taking phenformin because of persistent headache The mean weight loss during the two periods on phenformin was 2 7 kg/month and 2 9 kg/month respectively On 500 kcal/day she lost weight faster than when on phenformin (6 0 kg/month) but was distressed by painful sensa tions of hunger which forced her to stop banting.

The weight and the weight changes the con centrations of FFA and glycerol in plasma, and the acetone concentration in the alveolar air are given in *Table I for the first series* and in Table II for the second series

It will be seen that in the first series the mean weight reduction after four weeks on placebo was 0 8 kg and on phenformin 1 3 kg The mean individual weight reduction on phenformin was 0 5 kg more than that on placebo This difference was not statistically significant ($t = 1\ 42$ $n = 13$ $0\ 1 > p > 0\ 05$) In the second series there were no differences in the weight changes at all either on placebo or phenformin In both series the mean glycerol concentration was lower after phen formin than after placebo Although the glycerol concentrations were higher in the second series the mean values of the individual differences were similar in both series and the results have there fore been pooled The mean reduction after phen forming administration was 0 022 m moles/l ($t = 2\ 67$ $n = 33$ $p = < 0\ 01$)

Table I *Series no 1*

Case no	Age (y)	Weight (kg)	Weight changes kg — After 4 weeks on — Phen formin	Placebo	Glycerol m moles/l — After 2 weeks on — Phen formin	Placebo	Free fatty acids m moles/l — After 2 weeks on — Phen formin	Placebo	Acetone µg/l alveolar air — After ? weeks on — Phen formin	Placebo
1	52	66 0	−0 5	0 5	0 055	0 075	0 26	0 30	1 14	0 40
2	52	71 4	−1 9	+1 0	0 106	0 064	0 72	0 52	0 12	0 68
3	47	72 7	0 9	+1 0	0 080	0 051	0 58	0 30	0 90	0 57
4	53	69 0	0	0	0 103	0 136	0 44	0 45	0 90	1 80
5	23	92 2	−2 2	−1 0	0 122	0 194	0 60	0 65	0 45	—
6	51	87 1	1 9	−3 1	0 059	0 1 0	0 47	0 39	1 00	1 37
7	18	59 8	−1 6	−1 7	0 051	0 103	0 66	0 81	0 80	0 80
8	24	81 0	−1 3	−0 4	0 051	0 062	0 49	0 48	0 53	0 33
9	52	62 5	−1 3	−0 6	0 089	0 140	0 69	0 68	0 84	1 05
10	38	66 7	−1 2	+0 5	0 058	0 056	0 42	0 37	4 28	—
11	49	105 4	−1 0	−1 1	0 070	0 084	0 61	0 34	0 35	1 20
12	53	58 3	−0 9	−4 3	0 167	0 212	0 84	1 09	0 6?	1 71
13	45	85 7	−2 2	−2 4	0 079	0 086	0 71	0 63	2 55	1 73
Mean	42 9	75 2	−1 3	−0 8	0 084	0 106	0 58	0 54	0 96	1 06
S E. of mean ±	3 6	3 93	0 18	0 36	0 010	0 015	0 043	0 064	0 196	0 164
S D ±	12 8	14 2	0 63	1 25	0 034	0 052	0 16	0 23	0 71	0 54

Table II Series no. 2

Case no	Age (y)	Initially weight kg	Weight changes kg: After 4 weeks on Phenformin	Weight changes kg: After 4 weeks on Placebo	Glycerol m moles/l: Initially	Glycerol m moles/l: After 4 weeks on Phenformin	Glycerol m moles/l: After 4 weeks on Placebo	Free fatty acids m moles/l: Initially	Free fatty acids m moles/l: After 4 weeks on Phenformin	Free fatty acids m moles/l: After 4 weeks on Placebo	Acetone µg/l alveolar air: Initially	Acetone µg/l alveolar air: After 4 weeks on Phenformin	Acetone µg/l alveolar air: After 4 weeks on Placebo
Fasting													
1	50	104 3	−0 7	+1 2	0 117	0 089	0 704	1 01	0 56	0 99	2 78	0 68	0 32
2	55	145	+3 6	+4 0	0 0	0 240	0 348	0 56	0 54	1 01	—	0 72	1 10
3	45	81 6	−2 6	+0 9	0 074	0 056	0 095	0 61	0 41	0 32	0 86	0 34	0 43
4	59	173 7	−2 4	−2 5	0 132	0 164	0 185	1 08	1 28	1 27	2 50	1 38	1 28
5	49	88 1	+1 6	−1 6	0 111	0 059	0 053	0 62	0 22	0 46	0 75	0 55	1 11
6	56	138 4	−1 3	−2 1	—	0 294	0 205	—	1 31	1 17	2 30	1 90	1 04
7	65	89 1	−4 3	−0 6	0 088	0 074	0 077	0 67	0.55	0 47	—	0 70	2 23
8	49	116 5	+2 4	0	0 198	0 197	0 304	0 0	0 86	1 46	0 54	0 65	1 08
9	42	121 0	+1 4	−2 1	—	0 075	0 074	—	0 51	0 58	—	1 10	0 36
10	37	8 5	+0 2	+1 2	—	0 064	0 036	—	0 62	0 74	0 43	1 19	0 21
11	19	90 3	−0 3	+0 2	0 063	0 039	0 065	0 50	0 34	0 47	1 10	0 54	2 30
12	53	90 7	−0 7	+0 2	—	0 147	0 064	—	1 77	0 40	—	1 10	0 47
13	64	115 8	−1 1	+7 3	0 156	0.12	0 059	0 68	0 61	0 40	0 58	0 72	0 29
14	43	117 8	+0 7	+0 9	0 057	0 065	0 107	0 35	0 51	0 74	1 53	0 54	0 90
Mean	49	107 5	−0 46	+0 14	0 172	0 120	0 138	0 66	0 69	0 73	1 34	0 94	0 94
S E of mean ±	3 17	5 57	0 56	0 51	0 018	0 021	0 0 7	0 017	0 095	0 103	0 280	0 143	0 180
S D ±	11 86	20 89	2 10	1 83	0 053	0 079	0 096	0.25	0 36	0 39	0 885	0 533	0 672
Non fasting													
15	52	91 6	0 8	−0 8	0 150	0 170	0 180	0 64	0 76	0 70	1 35	1 20	1 57
16	64	84 9	0	0 2	0 136	0 090	0 141	0 57	0 47	0 54	0 37	0 61	0 31
17	51	90 0	0	0	0 141	0 073	0 134	0 94	0 21	0 72	1 00	0 21	0 50
18	47	87 4	0 8	1 0	0 145	0 728	—	0 37	0 77	—	0 92	1 08	1 30
19	6	71 6	+1 1	0 9	0 084	0 081	0 703	0 97	0 43	0 59	2 05	0 68	0 52
0	55	100 8	3 6	1 9	0 067	0 134	0 091	0 40	0 84	0 74	0 43	2 15	0 58
21	50	81 0	1 1	0 3	0 077	0 089	0 064	0 49	0 51	0 46	0 6	0 64	0 23
Mean	49	86 7	0 03	0 13	0 114	0 174	0 136	0 63	0 57	0 54	0 98	0 94	0 71
S E of mean +	4 69	3 44	0 63	0 38	0 014	0 072	0 021	0 092	0 086	0 071	0 22	0 735	0 189
S D +	1 79	8 41	1 67	1 01	0 037	0 058	0 052	0 4	0.22	0 17	0 586	0 676	0 500
Total													
Mean	49	100 6	0 17	+0 05	0 118	0 1 2	0 137	0 65	0 65	0 68	1 19	0 94	0 86
S E of mean +	55	4 9	0 42	0 35	0 011	0 015	0 018	0 057	0 069	0 076	0 189	0 1 0	0 135
S D +	11 66	19 7	1 88	1 54	0 047	0 069	0 082	0 3	0 31	0 34	0.760	0 535	0 602

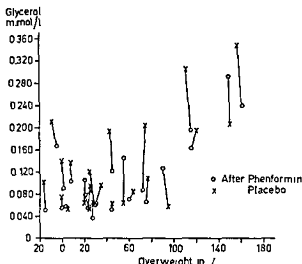


Fig 2 The correlation between the plasma glycerol con centration and the relative overweight in both series

When comparing the placebo period with the phenformin period there were no consistent changes in either FFA or the acetone concentra tion

As there did not appear to be a linear cor relation between glycerol and overweight (Fig 2) or FFA and overweight statistical significance was tested by rank correlation In the first series there were no significant correlations between the concentration of glycerol and overweight or FFA and overweight whereas in the second series there were Here as previously mentioned over weight was considerable When the study was begun the coefficient of correlation was signifi cant at the 5% level correlating glycerol to over weight and FFA to overweight When the patients were given phenformin and placebo the corre sponding values were $r = 0\ 72$ $n = 16$ $p < 0\ 001$ and $r = 0\ 61$ $n = 16$ $p < 0\ 01$ respectively FFA versus overweight was significant at the 1% level in both periods

There was a linear correlation between FFA and glycerol as shown in Fig 3 The coefficient of correlation for the whole material i e in both series on placebo and on phenformin was 0 74 $n = 67$ $p < 0\ 001$

DISCUSSION

Phenformin alone has no influence on the weight of subjects with resistant obesity as evidenced by the second series These patients had previously been given various forms of treatment and there was no general weight reducing effect due to the fact that they were checked by a physician and their weight regularly controlled etc In the first series consisting mainly of moderately obese women the placebo effect resulted in a mean weight reduction of 0 8 kg in one month The additional weight loss when on phenformin was not statistically significant although the mean weight reduction was 1 3 kg However if this observation is viewed in conjunction with the well

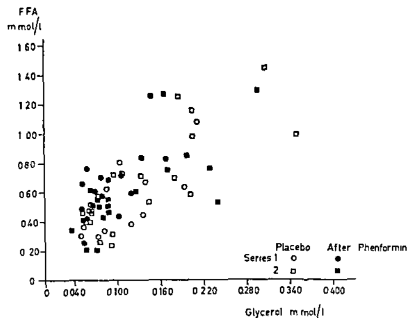


Fig 3 The correlation between the plasma concentrations of free fatty acids and glycerol in both series

documented case in Fig 1 the conclusion seems warranted that with the given dose phenformin has a weight reducing effect in cases of moderate obesity

Roginsky and Barnett (12) observed a weight loss, in patients on phenformin of the same order of magnitude as that obtained in the present study although their results were only significant for patients with a family history of diabetes

The reducing effect observed also agrees with the preliminary reports of Burstein et al (2) and with those of Liebermeister et al (10) who found a mean weight loss of 1 4 kg in 35 obese patients after six weeks on a dosage of 200 mg of butyl biguanide Moreover this effect is in keeping with Pedersen s (11) finding that weight loss increased when patients on 1250 calories were given 3–400 mg of dimethyl biguanide hydrochloride (metformin) Pedersen emphasized the need of a high dosage and it is possible that the patients in the second series who were stouter should have received more phenformin for a comparable dosage When a weight reducing effect is observed in obese subjects given phenformin this seems to be of about the same order of magnitude as that in obese diabetics (5)

On caloric restriction leading to weight loss the acetone concentration in the alveolar air increases (13) The absence of changes in the acetone concentration in series II may be accounted for by the absence of weight changes In series I the weight changes after placebo were also small whereas a certain increase in the acetone concentration would have been expected on phenformin judging by the weight loss This lack of increase in the acetone concentration may be explained either by a reduced tendency to ketosis in obese subjects on caloric restrictions as described by Kekwick et al (6) or by an antiketogenic effect due to phenformin itself

No changes in the concentration of FFA were observed after phenformin which agrees with the findings of Burstein et al (2) and of Liebermeister et al (10) The latter found no changes in the concentration of plasma glycerol whereas in the present study a statistically significant ($p < 0\ 01$ $n = 23$) decrease was observed during phenformin treatment A more detailed comparison with the results of Liebermeister et al is not possible owing to the brevity of their report

For reasons discussed by Havel (4) and Steinberg (15) the rate of lipid mobilization in adipose tissue is more adequately illustrated by the plasma concentration of glycerol than by FFA The present results may therefore be explained by a reduction in the rate of lipid mobilization in the morning due to phenformin on the assumption that phenformin does not increase the glycerol elimination rate

ACKNOWLEDGEMENT

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REFERENCES

1 Bergqvist N Lakartidn 63 751 1966
2 Burstein J Lindell K Nikiforow R Jakobson, T & Nikkila E A Diabetologia 2 33 1966
3 Grodsky C M Karam, J H Pavlatos F Ch & Forsham P H Metabolism 1 78 1963
4 Havel R J Ann N Y Acad Sci 131 9 1965
5 Iversen M Englund F Wollesen F Hansen F & Andresen P Diabetologia 33 1966
6 Kekwick A Pawan G L S & Chalmers T M Lancet 2 1157 1959
7 Krall L P & Bradley R F Postgrad med J 37 75 1965
8 Laurell S & Tibbling G Clin chim Acta 13 317 1966
9 — Clin chim Acta 16 57 1967
10 Liebermeister H Ruenau er R Gruneklee D Schilling W Jahnke K & Daweke H Diabetologia 2 208 1966
11 Pedersen J Acta endocr 49 479 1965
12 Roginsky M S & Barnett, J Amer J clin Nutr 19 223 1966
13 Rooth, G & Östenson S Lancet 2 1102 1966
14 Schwartz, M J Mirsky S & Schaefer L E Metabolism 15 808 1966
15 Steinberg, D Jr In Fat as a tissue McGraw Hill New York 1964

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PENTAERYTHRITOLTETRANICOTINATE (PERYCIT®) IN THE TREATMENT OF HYPERCHOLESTEROLAEMIA

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Abstract For more than ten years we have tried to attack the problem of hyper holesterolaemia and hyperlipaemia using dietary treatment as well as various drugs

Nicotinic a id given per os has been shown to lower serum cholesterol. A rather high frequency of side effects has motivated trials of derivatives with l ss marked side effects.

Pentaerythritoltetranicotinate is equal in effect to nico tinic acid but produces fewer side effects During treat ment periods of up to six years no harmful influence was seen.

A comparison of the three substances clofibrate triiodo-thyreopropionic acid and pentaerythritoltetranicotinate in dicates that clofibrate is effective in lowering cholesterol in most cases No effect on peripheral circulation has been observed and at least during short periods it does not combat angina pectoris

Triiodothyreopropionic acid is particularly effective in cases with a tendency to hypothyreodism Its availability is reduced by a tend ncy to produce or increase angina pectoris. Pentaerythritoltetranicotinate Perycit® is equal in effect and better tolerated than nicotinic acid but some patients may not tolerate it either Perycit is always effective in lowering cholesterol it increases the peripheral circulation and has a favourable effect on angina pectoris

The purpose of this paper is to report on the experiences gained from clinical trials with penta erythritoltetranicotinate (Perycit) in tablet form

Chemistry

Pentaerythritoltetran cotinate (Pery it®) trial name 8 AL AB Bofors Nobel Pharma Sweden, is an ester of nicotinic acid The drug is spa ingly soluble in water 0 034 mg/ml (37 C) but more easily soluble in gastric juice 16.5 mg/ml (37 C) (7)

Pharmacology

LD_{50} orally in mice is > 10 000 mg/kg. The drug has passed toxicity tests performed on rats and dogs Tests for possible teratogenic effects in rats and rabbits proved negative for therapeutic doses.

Compared with the same dosage of nicotinic acid 8 AL produces less flushing in the guinea pig (neck and ear) In rabbits 8 AL has been shown to have a better pro tective effect, than nicotinic acid against arteriosclerosis induced by die s rich in fat and cholesterol (9)

First Trial (Long Term)

MATERIAL AND METHODS

Nicotinic acid was first tried on 15 of the patients Two of them could tolerate 1 g three times daily but 13 immediately de lared that the drug produced such an intense flush often accompanied by dizziness that it had to be withdrawn. When given 8 AL 1 g × 3 three of these 13 patients stated that they could not tolerate this prepara tion either In the remaining ten patients similar but milder reactions occurred as with nicotinic acid and they consid red 8 AL tolerable

Seventeen patients including the ten just mentioned rece ved treatment with 8 AL for not less than two month Treatment was temporarily stopped for one to two months Today six of the patients have been on 8 AL for five to six years In addition to hypercholesterolaemia three of the patients had hyperlipaemia six had earlier cardiac infarctions and 11 suffered from angina pectoris w thout electrocardiographic confirmation of coronary in sufficiency All the patients were prescribed a low fat diet with a high ratio of polyunsaturated fatty a ids The patients followed the instructions but admitted to some transgressions especially at Christmas

During the whole cours of treatment all th patients were regularly examined at first once a week later monthly to quarterly with blood tests including white cell differential and thrombocyte counts serum tests for liver fun t on cholesterol and protein ECG etc

RESULTS

Less than 3 g daily of 8 AL did not lower the serum cholesterol level permanently With a dosage of 3–6 g daily a permanent fall was obtained The decrease occurred after one to two weeks of therapy If 8 AL was withdrawn the cholesterol returned to its original level in two weeks This occurred even after several years of treatment.

Fig. 1. Serum cholesterol values with different kinds of therapy.

When compared with nicotinic acid, aluminium nicotinate or clofibrate in equivalent doses the results were approximately the same. Inositol pentanicotinate, on the contrary, did not seem to influence the cholesterol level. Some patients preferred clofibrate to 8 AL because they had less side effects, and some preferred 8 AL because they had less angina pectoris and better working capacity with this therapy. Leucopenia caused by clofibrate (12) has not been observed in this series.

Triiodothyreopropionic acid (23) was given in dosages up to 2 mg a day. A favourable effect was produced initially, but in most cases the dosage needed to reduce serum cholesterol caused an increase of angina pectoris. Combination therapy consisting of 8 AL and clofibrate or triiodothyreopropionic acid offered no advantages. Fig. 1 shows an example of the changes in cholesterol values as a result of different kinds of therapy and dosages over a long period (patient no. 13). Comparable curves were obtained for all patients, but are not included for reasons of space.

Table I. *Distribution of cholesterol mean values for patients on dietary but before drug treatment*

mg/100 ml	275–300	300–400	>400
No. of pats.	1	10	6

Laboratory investigations

No injurious effect on blood elements or liver function was seen, except for a temporary rise of SGOT to 54 units in a patient who had a period of heart insufficiency. During regular controls none of the patients showed glycosuria. Glucose tolerance tests were not carried out.

During the trial it was decided to extend the liver function tests to include bromsulphalein excretion. Five patients had pathologic retention values (Table II), which, in three cases, led to further liver function tests and liver biopsy. No other signs of possible liver damage were observed. When the bromsulphalein excretion test was repeated, the results seemed to be independent of the treatment, and consequently, the test was considered to be without clinical significance during treatment with nicotinic acid derivatives. Parsons (3, 18) and Schreiber (2) reached similar conclusions. Furthermore, during the injection of bromsulphalein one patient reacted so strongly by vomiting and fall in blood pressure that the test could not be completed. As severe reactions, including fatalities, have occurred (4), it was decided to discontinue the bromsulphalein excretion tests.

All the patients who, according to Table II, had raised bromsulphalein retention values, have been treated with 8 AL for 18 months after the retention test, and none has shown signs of liver damage.

Side effects

Flushing was recorded in all the patients, but was less marked than with nicotinic acid. It was possible either to eliminate or to decrease flushing by taking the tablets after a meal and/or by dividing the daily doses into several smaller doses. In most cases, however, the attacks of flushing diminished after a fortnight. Flushing usually occurred after 15–60 minutes, and disappeared within ten minutes. In nervous and unaccustomed patients flush-

Table II *Results of bromsulphalein retention tests and liver biopsies*

Pat no	Sex	Age	Bromsulphalein retention ( )	8 AL dose (g)	Period of treatment	Liver biopsy
3	♂	70	9 4	4–5	6 mo	
3	♂	70	6 0	4–5	7 mo	No signs of marked liver damage
3	♂	70	22 8	0	2 d	
7	♂	44	4 3	3	8 mo	
12	♀	41	16	6	4 mo	
12	♀	41	4 1	6	4½ mo	No changes of the kind observed following damaging medication
12	♀	41	1	0	3 w	
12	♀	41	2	0	2 mo	
13	♂	50	1	4	5 mo	
14	♀	49	10 8	3	2½ mo	
14	♀	49	5 6	3	6 mo	No pathological signs
15	♂	58	8 8	4	2 w	
16	♂	57	6 3	3	18 mo	
17	♂	62	1 1	3	13 mo	
18	♂	59	7 6	4	2 mo	
19	♀	60				Reaction (cf text)

ing could be accompanied by dizziness Some patients remarked that if they ate something acid usually oranges between meals this could promptly cause flushing even some hours after taking the tablets

Gastric complications

Usually slight nausea and occasionally slight anorexia occurred periodically when large doses were administered for a long time These complications were comparatively easy to treat with antacids sometimes in combination with anticholinergics Gastric symptoms were most marked in patients with an earlier tendency to gastritis

Two patients had a myocardial infarction during treatment After recovery treatment with 8 AL continued for more than two years In neither case was the 8 AL therapy considered to have caused the infarction

Second Trial

MATERIAL AND METHODS

The second group studied consisted of 12 outpatients with hypercholesterolaemia and angina pectoris (Table III) Two patients (1 and 3) from the long term trial were temporarily included

The trial was divided into four periods

1 A control period of four weeks with the same dietary instructions as in the long term trial and no further therapy
2 A treatment period of six weeks during which 8 AL was added
3 A second control period of six weeks without this drug
4 A further treatment period of six weeks

Table III *Distribution of patients*

Pat no	Sex	Age	Weight (kg)	Chol (mg)	Diagnosis other than hypercholesterolaemia and angina pectoris
1	♀	59	79	352	
2	♀	48	7_	333	Benign essential hypertension
3	♂	70	64	390	Myxoedema
4	♀	60	67	339	Spondylos deformans hypertension cystitis
5		61	83	347	Benign essential hypertension Diverticulitis coli
6	♀	55	87	383	Benign essential hypertension Myxoedema
7	♂	44	68	388	Hyperlipaemia Myocardial infarction
8	♀	53	48	431	Hyperlipaemia Acute myelitis
9		36	69	425	Hyperlipaemia
10	♂	58	77	355	Hyperlipaemia xanthoma (olecranon region)
11	♀	65	4	349	Hyperlipaemia Raynaud s phenomenon
12	♀	41	66	369	Hyperlipaemia Coronary insufficiency Intermittent claudication

Mean values from control period 1

Table IV *Mean values of cholesterol in mg*

Pat no	Control period 1	Treatment period 1	Control period 2	Treatment period 2
1	352	243	311	260
2	333	298	319	281
3	390	350	433	304
4	339	310	361	321
5	347	316	359	302
6	383	417	455	337
7	388	229	315	264
8	431	294	329	251
9	4 5	311	415	3₂9
10	355	216	331	—
11	394	265	—	—
12	369	360	—	—
Mean[b] ±S E	376±12	308±18	366±18	294±11

t test on individual differences[b]

Control period 1 v treatment period 1 $p < 0\ 05$
Control period 2 v treatment period 2 $p < 0\ 001$
Control period 1 v control period 2 N S
Treatment period 1 v treatment period 2 N S

[a] Dosage only 1 5 g daily
[b] Patients no 1–9

In some instances the second control period was extended without affecting the trial The dosage of 8 AL was 3 g daily

Serum cholesterol determinations (6 11) were made every second week thus providing three values per period Liver function was followed by serum bilirubin levels alkaline phosphatases thymol turbidity and SGOT Leucocyte and platelet counts as well as prothrombin estimations were made

RESULTS

The effect of 8 AL on the serum cholesterol is shown in Table IV and Fig 2 With adequate dosage a statistically significant lowering of the serum cholesterol level was obtained Seven patients were not on diet when the trial started Before the first control period they had a mean cholesterol value of 420±22 mg% which decreased to 378±16 mg % during this period

All patients reported that their angina pectoris was both less severe and less frequent and five of them considered that the sensation of heaviness in their legs was milder when taking 8 AL

Studies have shown that nicotinic acid is more rapidly eliminated from the body than 8 AL (14) The advantage of slow elimination is that a lower dosage ought to produce an adequate lowering of the cholesterol level This may be illustrated by patient 12 who prior to the present investigation was given in a trial nicotinic acid in increasing doses up to 6 0 g daily whereby her cholesterol level fell from 370 mg/100 ml to 291 mg/100 ml When a steady cholesterol level had been established a change to an equal dose of 8 AL resulted in a further fall to 212 mg/100 ml A parallel lowering of her serum lipids was obtained These results were reproduced with a somewhat lower dose During this trial the patient complained of marked gastric symptoms when on nicotinic acid which were alleviated only to a minor degree by antacids When she received 8 AL her symptoms rapidly subsided While on 8 AL therapy she also reported improved walking capacity

Laboratory investigations

Laboratory results are shown in Table V Patient 1 showed a rise in serum alkaline phosphatases These were rapidly normalized after the end of the trial and were probably elevated owing to gallbladder disease Patient 12 showed already before the trial a positive thymol turbidity reaction of 0 14 which remained unchanged Triglycerides were also determined but not regularly they followed the variations in serum cholesterol

Side effects

Side effects were of the same kind and frequency as in the long term trial Thus flushing was recorded in all the patients except no 12 In one case flush and in two hyperacidity caused therapy to be discontinued

DISCUSSION

The effect of nicotinic acid in lowering serum lipids is amply documented (1 2 3 9 15 16 21) Three g or more daily of 8 AL also produce a *reduction of the same magnitude in the serum*

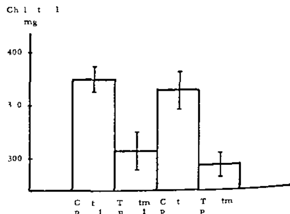


Fig 2 Cholesterol values (mean ±S E) for patients no 1–9

Table V *Values of different clinical and laboratory tests during the investigation*

Pat no	Blood pressure C	Blood pressure T	BMR C	BMR T	SGOT C	SGOT T	Icterus index C	Icterus index T	Thymol turbidity C	Thymol turbidity T	Alkaline phosphatase C	Alkaline phosphatase T	Leucocytes 10³ C	Leucocytes 10³ T	Platelets 10 C	Platelets 10 T	Prothrombin C	Prothrombin T
1	155/90			±0	26	26	1 4	1 1	0 03	0 05	7 4	12 7	2 9	4 3	266	196	96	
	160/110	170/110			20	20	1 5	1 6	0 04	0 06	4 0	4 0	4 4	4 3	288	234	88	77
3	160/80		−1		15	30	1 5	1 3	0 07	0 12	1 6	2 0	5 8	4 3	168	222	105	79
4	130/90		+2		17	12	1 3	1 4	0 03	0 02	10 0	8 7	6 8	10 5	350	290	98	91
5	150/85	140/90	−4		28	17	1 5	1 5	0 03	0 04	7 9	5 8	7 7	5 4	350	346	104	80
6	170/100		+6		19	14	1 6	1 3	0 01	0 02	9 9	9 9	4 4	4 5	266	236	116	87
7	145/100		−1			22		1 8	0 06	0 05	5 8	6 2	4 4	3 8		208		
8		170/110	+12		12	17	1 5	1 8	0 05	0 06	5 2	7 5	3 9	3 6	170	224		
9	140/80	145/90	−2		26	34	1 5	1 7	0 07	0 09	8 6	8 3	4 4	4 2	244	⌞72		
10	135/80		−14				1 6	1 3	0 04	0 03	4 5	7 9	5 6					
11	150/80		−4		12	19	1 5		0 01	0 02	3 6		5 3					
12	140/85		+8			14	1 6	1 3	0 14	0 16	4 1	4 8			264			

C = values towards the end of a control period
T = values towards the end of a treatment period

cholesterol As with nicotinic acid this decrease is reversible when therapy is withdrawn

Nicotinic acid in the requisite doses for an effective lowering of serum cholesterol i e 3 g a day or more (16) causes such pronounced side effects at least in the earlier stages of treatment that the patients often refuse to continue the therapy (5 13 17 19) The side effects can be reduced by taking the drug after meals and by administering successively increasing doses According to the patients the side effects of 8 AL were considerably milder and more tolerable

Flush is the most troublesome of the side effects Generally it subsides after a short period of treatment but some patients insist that it is impossible to raise the dose beyond a certain limit—commonly 0 5 g each time It is possible to achieve higher dosage by dividing the dose into several small doses Stomach complaints such as generally occur in connection with gastritis can be coped with by administration of antacids or/and anticholinergic drugs

Modifications of nicotinic acid with a prolonged effect may be associated with liver damage (10 20) Regular controls of blood and liver function (bilirubin thymol turbidity alkaline phosphatases and SGOT) have not shown any serious side effect even in patients who have taken 8 AL 3 g a day for 5–6 years

REFERENCES

1 Achor R. W P & Berge K. G Med Clin. N Amer 4 871 1958
2 Altschul R., Hoffer R. & Stephen, J D Arch Biochem 54 558 1955
3 Altschul R. Niacin in vascular disorders and hyper lipaemia Thomas Springfield 1964
4 Astin T W Brit med J 11 408 1965
5 Berge K. G Geriatrics 16 416 1961
6 Bjorntorp P & Cramer K Scand J clin. Lab Invest 13 434 1961
7 AB Bofors Nobel Pharma Unpublished
8 Brattsand R. & Lundholm L To be published
9 Carlson L A & Oro L Lakartidn. 63 4?81 1966
10 Christensen N A Achor R W P Berge K. G & Mason H L J.A.M.A 177 546 1961
11 Cramér K. & Isaksson B Scand J clin Lab Invest 11 ?13 1959
12 Engstrom J Acta med scand 180 519 1966
13 Gurian H & Adlersberg, D Amer J med Sci 237 12, 1959
14 Harthon L & Sigroth K Unpublished data
15 Ljunggren H Grissler J & Probst, F Svenska Lak. Tidn 58 653 1961
16 Paoletti R. (ed) Lipid pharmacology Academic Press New York 1964
17 Parsons W B Jr J.A.M.A 173 1466 1960
18 — Arch intern Med 107 639 1961
19 — Arch intern Med. 107 653 1961
20 — J Amer Geriat Soc 10 850 196?
21 Rivin A R. Calif Med 96 ⌞67 196?
22 Schreiber F K Klin. Wschr 42 1070 1964
23 Welin, G To be published

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THE EFFECT OF PROPRANOLOL ON ECG IN ANGINA PECTORIS AND ORTHOSTATIC TACHYCARDIA[1]

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Abstract The effect of intravenous infusion of 5 mg propranolol on the electrocardiogram at rest during and after work on a bicycle ergometer was studied in 76 patients with either typical or questionable coronary heart disease or with orthostatic tachycardia In the last group electrocardiograms were also registered in the upright position In the patients with orthostatism propranolol elicited a normalization of the S-T and T changes in standing position and during work. Propranolol had no demonstrable effect on the electrocardiographic picture during work in patients with typical coronary disease though it did elicit a significant reduction of the depressed S-T and T segments three minutes after work This observation may be connected with the possibility that the cardiac work and hence the degree of ischemia was less in the work test after propranolol compared with that before In the patients with questionable coronary disease propranolol elicited a reduction of the electrocardiographic changes both during and after work Such an effect would be in line with the presence of an increased activity in the cardiac sympathetic nerves and possibly also with a moderate degree of coronary sclerosis

Attempts to differentiate between a vegetative and an organic etiology of electrocardiographic changes using sympatholytic agents were made at an early stage (2 17 21) Besides blocking alpha receptors however the substances available at the time also stimulated adrenergic receptors making it difficult to assess the results Interest in this type of differential diagnostic study has revived with the advent of substances chiefly propranolol that specifically block beta adrenergic receptors and thus the adrenergic functions of the heart Thus it has been shown that S T and T changes connected with orthostatic reactions can be suppressed by treatment with propranolol (7 13 18) This observation was held to confirm the previous assertion that electrocardiographic changes of this type indicate an elevated sympathetic tonus in the heart

Propranolol has also been used to differentiate electrocardiographic changes caused by functional or organic cardiac disorder observed after effort It has thus been found that in patients with coronary disease propranolol or its predecessor pronethalol does not affect S T and T changes that arise during work (7 22) or after work (23) Furberg (7) reported however that the electrocardiographic changes became normalized more rapidly after the end of work when patients with coronary disease were given propranolol He interpreted this to mean that the shape of the ST T segment after the end of work is influenced not only by ischemic changes but also by changes in the activity of cardiac sympathetic nerves

The present investigation was an attempt to evaluate the use of propranolol for the assessment of ST T changes on exercise electrocardiograms The investigations concerned the effect of the substance upon electrocardiographic changes during and after work in patients with clinically established or suspected coronary insufficiency as well as in individuals with orthostatic reactions

METHODS

Electrocardiography

Electrocardiograms were recorded at rest in both the supine and the standing position using unipolar and bipolar extremity leads as well as the following chest leads CR CR_2 CR CR_4 and CR Only the chest leads were used during work, the indifferent electrode being shifted from the right arm to the forehead (CH) This change does not usually affect the pattern of the electrocardiogram (11)

The electrocardiograms were coded according to Black

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[1] A report on an introductory study was presented at the Swedish Medical Convention (Medicinska Riksstämman) in Stockholm 1964

Table I *Heart rate (means* $\pm$ S E) *at rest during and after exercise performed before and after intravenous infusion of 5 mg propranolol*

The data given during work (before and after propranolol) were obtained at identical times during the same work load The heart rates just before the end of work were not always obtained at identical work loads in each patient before and after propranolol (see Procedure)

	Rest		Work (?00–300 kpm/min)		Work (400–600 kpm/min)		Just before the end of work		3 min after work	
Patients	Before	After	Before	After	Before	After	Before	After	Before	After
With typical coronary	72 4	63 5	100 2	88 3	127 8	109 5	133 8	118 2	81 2	73 8
heart disease ($n = 12$)	$\pm$2 7	$\pm$2 1	$\pm$2 7	$\pm$2 5	$\pm$3 0	$\pm$2 7	$\pm$4 8	$\pm$3 5	$\pm$2 8	$\pm$2 2
With questionable coronary	76 5	66 0	112 7	95 5	147 0	119 8	148 5	131 4	85 9	76 1
heart disease ($n = 6$)	$\pm$5 9	$\pm$3 5	$\pm$6 6	$\pm$8 1	$\pm$16 6	$\pm$13 7	$\pm$11 4	$\pm$13 1	$\pm$6 0	$\pm$5 1
With orthostatic	75 0	65 6	114 8	91 8	140 7	112 1	153 6	134 0	92 5	78 1
tachycardia ($n = 8$)	$\pm$2 3	$\pm$2 1	$\pm$5 3	$\pm$3 3	$\pm$6 1	$\pm$3 3	$\pm$9 2	$\pm$7 3	$\pm$6 6	$\pm$4 6

burn et al (3) as modified by Astrand (1) and with two further modifications whereby IV 7 stands for isoelectric or elevated S-T with normal configuration and an additional category V 4 is added under V The code employed was as follows

IV 1 S T J depression 1 mm or more and S-T segment horizontal or downward sloping

IV 2 S-T J depression 0 5–0 9 mm and S T segment horizontal or downward sloping

IV 3 No S-T J depression as much as 0 5 mm but S T segment sloping down to 0 5 mm or more below P R baseline

IV 4 No S T J depression as much as 0 5 mm S T segment horizontal or downward sloping but not reaching 0 5 mm below P R baseline

IV 5 S-T J depression 1 mm or more with normal configuration of S-T segment

6 S-T J depression 0 5–0 9 mm with normal configuration of S T segment T wave items (when R amplitude = 5 mm or more in aVL and QRS mainly upright in aVF)

IV 7 Isoelectric or elevated S T with normal configuration

V 1 T amplitude = minus 5 mm or more (I II CR – CR_7)

V 2 T amplitude = minus 1 to 5 mm (I II CR_4–CR_7)

V 3 T wave flat or slightly diphasic negative phase less than 1 mm (I II CR –CR_7)

V 4 T amplitude positive 2 mm or more (I II CR_{4-})

PATIENTS

Twenty-six patients participated in the investigation All showed ST T depressions on electrocardiographic recordings during exercise They were divided into three groups

Group 1 (patients with typical coronary heart disease) Twelve patients (10 men and 2 women) had symptoms of effort angina with pain located retrosternally or to the left side of the chest radiating to the left arm, and with relief of pain at rest and after treatment with nitroglycerin

Group 2 (patients with questionable coronary heart disease) Six patients (3 men and 3 women) experienced no pain on exercise However their electrocardiographic changes during exercise were similar to those in group 1 The possibility that these patients had a coronary disease could therefore not be ruled out

Four of them (2 men 54 and 57 years and two women 32 and 47 years) had pain located to the left side of the chest (in two of them radiating to the left arm) which could appear at any time with no relation to exercise In two of them coronary angiography showed no abnormality except a suspected narrowing of a vessel in one of them Furthermore this patient had almost consistently atrial fibrillation The remaining two patients had no history of pains They included one man 51 years with artherosclerotic changes of the aorta as shown at angiography and one woman 58 years with diabetes mellitus

Group 3 (patients with orthostatic tachycardia) The remaining eight patients (5 men and 3 women) had no angina pectoris However they developed extensive tachycardia and electrocardiographic ST T depression during 8 min standing

The ages (means $\pm$ S E.) of the patients were 51$\pm$2 3 in group 1 50$\pm$4 0 in group 2 and 35$\pm$3 6 years in group 3

PROCEDURE

The work test was performed on an electrically braked bicycle ergometer (9) The load was increased successively the initial level being 200 kpm/min for women and 300 (in a few cases 200) kpm/min for men

Two identical studies were performed within a week on each individual The studies were made on different days in the case of eighteen patients in the other eight they were performed on the same day with at least two hours rest between the exercise tests (two of these nine patients belonged to group 1 two to group 2 and four to group 3) On each occasion electrocardiograms were recorded at rest in the supine position and also in the case of patients with orthostatism after 8 min standing The work test was then performed in the sitting position electrocardiograms being registered after 2 4 5 and 6 min work at each load as well as just before the end of work

The pr uent was then placed in the supine position and a further registration was made 3 m n later On the second occasion 5 mg propranolol (Inderal) was infused intra venously during 5 min into the supine patient (Inderal from Dr A Wagner AB Scanmeda Göteborg Sweden.) Further electrocardiograms were then registered 5 min after the end of the infusion followed by the orthostatic test and the exercise test with electrocardiographic record ings as on the first occasion The load was increased every sixth minute by 200 and 300 kpm/min in women and men respectively The work test was always continued until the patient experienced angina pectoris or fatigue This meant that th total amount of work performed was not always the same before and after propranolol The second work test was completed about half an hour after the infusion of propranolol

RESULTS

Heart Rate

As will be seen from Table I propranolol elicited a fall in heart rate at rest in all groups as well as a reduced rise in heart rate during work The heart rate measured at the end of work was also lower after propranolol even though the total amount of work performed was somewhat larger Furthermore the heart rate 3 min after the end of work was lower after propranolol The patients with orthostatic tachycardia displayed during standing a lower increase in heart rate after pro pranolol than before the means ±S.E. being 102 3±5 1 before and 78 9±3 4 after propranolol ($p = < 0\,01$)

Electrocardiograms (Fig 1)

Group 1 (patients with typical coronary heart disease)

Before propranolol the S-T and T segments *at rest* showed only moderate deviations from the normal picture in all cases and the appearance of the S T segment was never classified below IV 4 Propranolol reduced these changes in a few cases but the difference was not significant for the group as a whole

During work all patients displayed a marked depression of the S T before propranolol in many cases combined with a flattened or diphasic T The electrocardiographic picture was not in fluenced by propranolol either when the com parison was made at the same work load or at the sam heart rate

After the end of work the S T changes recorded were significantly smaller after propranolol than before The T changes were al o reduced to some extent by p opranolol

Group 2 (patients with questionable coronary heart disease)

All the electrocardiograms registered *at rest* in this group displayed only very slight or no deviations from the normal picture

During work there was moderate to marked de pression of the S T segments while the T wave remained practically within normal limits The in fusion of propranolol was followed by less marked S T changes though the difference was not sig nificant

After work the S T depression before pro pranolol was of approximately the same magnitude as during work Propranolol resulted in an almost complete normalization (Fig 1)

Group 3 (patients with orthostatic tachycardia)

All the electrocardiograms in this group displayed normal or almost normal S T and T segments *at rest*

In the upright position all cases displayed de pression of the S T segment and a flattened or diphasic T (Fig 2) A normalization was registered throughout after propranolol the difference in the electrocardiographic reaction before and after the infusion being significant.

During work the electrocardiographic picture was always essentially similar to that recorded in the standing posture with a rapid normalization immediately after the end of work Propranolol normalized the electrocardiographic reaction dur ing work

Physical Work Capacity

In the group of patients with typical coronary heart disease the mean maximum load tolerated by the men was 600 kpm/min the duration of work at this load being on an average 3 min be fore and 5 min after propranolol For the women the average work capacity was 400 kpm min for 6 min before and for 3 min after propranolol These differences are not significant In the group of patients with questionable coronary heart disease the corresponding data for the men were 600 kpm/min for 5 min before and for 6 min after propranolol and for the women 400 kpm/min for 6 min before and 600 kpm/min for 1 min after propranolol These differences are not sig nificant either For the individuals with orthostatic ta hycardia the average maximal load tolerated before propranolol was 1200 kpm/min for 1 min

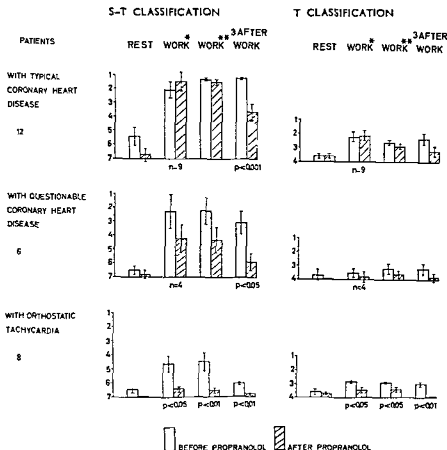


Fig 1 S-T and T classification of electrocardiograms at rest during work and 3 min after work in patients with 1 typical coronary heart disease 2 questionable coronary heart disease and 3 orthostatic tachycardia before and after intravenous infusion of 5 mg propranolol (means $\pm$ S E) S T classification 7 = normal configuration T classification 4 = normal configuration (see Methods)

* Comparison between the work load before propranolol that gave the same heart rate as the highest load after propranolol

** Comparison between *the same work load (highest* comparable load) before and after propranolol

for the men and 400 kpm/min for 6 min for the omen After propranolol the corresponding r ‥s were 1200 kpm/min for 2 min and 600 /min for 2 min Neither difference is significant

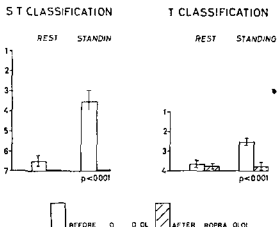


Fig S T and T classification of electrocardiograms at rest and standing in patients with orthostatic tachycardia before and after intravenous infusion of 5 mg propranolol (means $\pm$ S E) S T classification 7 = normal configuration T classification 4 = normal configuration (see Methods)

Subjective discomfort during work

In group 1 the physical working capacity was limited in all cases by unquestionable complaints of angina pectoris None of these patients reported any essential relief from the administration of propranolol One patient who had emphysema in addition to coronary insufficiency reported much more marked dyspnea during the work test after propranolol In group 2 the working capacity was limited by feelings of general fatigue or by uncharacteristic complaints from the chest Propranolol did not significantly affect the complaints in this group either Finally in the orthostatic group the limiting factor was fatigue chiefly in the leg muscles Three of these individuals reported a less pronounced sensation of fatigue at the same load after propranolol

DISCUSSION

In routine clinical work one is often faced with the problem of whether in the individual case electrocardiographic changes in the ST T segment

at rest or in connection with physical work reflect ischemic cardiac disease or whether they are of a more functional nature It seems difficult for theoretical reasons to determine the underlying cause of an ST T change solely from the appearance of the electrocardiogram The disturbance in the electrolyte balance at the cellular level which is probably the ultimate cause of the repolarization disturbance can thus be elicited by such widely different factors as ischemia drugs and hypokalemia Lepeschkin however has reported a flattened and depressed S T segment caused by an injury current of the subendocardial muscle fibres as a result of true coronary insufficiency (14) It has therefore been considered that such an S T change often combined with a diphasic T after work constitutes a criterion of latent myocardial ischemia (24) Reference has thus been made in this context to the similarity between this change and that which may appear at rest in connection with an attack of angina pectoris A good correlation has also been demonstrated between this type of electrocardiographic change and the mortality in coronary disease (16 20) On the other hand individuals with pronounced autonomic disturbances but not demonstrable cardiac disorder (referred to as vasoregulatory asthenia) may sometimes display a similar electrocardiographic picture during work with normalization immediately after the end of work (10) Also in coronary heart disease the ST T changes are most pronounced during work (4) From this it follows that one cannot always differentiate between organic and functional cardiac disorders simply on the basis of the type of electrocardiographic changes or the conditions under which these occur

It would therefore be valuable to have a substance that blocks the influence of the central nervous system upon the function of the cardiac muscle but which does not affect S T and T changes of organic origin The present results support the observation by Furberg (7) that drugs which elicit adrenergic betareceptor blockade may be of value for this purpose A clear electrocardiographic difference was thus demonstrated between patients with pronounced orthostatism and patients with coronary heart disease In the former group propranolol elicited a normalization of the S T and T changes in the standing position as well as during work whereas no electrocardiographic effect could be demonstrated during work in the group with coronary disease

It is particularly worth noting that propranolol significantly reduced the S T and T changes after the end of work in patients with typical clinical symptoms of angina pectoris This finding which agrees with Furberg s observation (7) indicates that in patients on acute or chronic beta adrenergic blockade the electrocardiographic analysis must refer to conditions *during* work when evaluating the S T and T regions in cases with suspected ischemic heart disease An analysis confined to electrocardiographic changes *after* work may thus give a false negative picture in such cases

It is not entirely clear why propranolol affects the electrocardiographic picture after the end of work but not during work in these cases According to Furberg (7) this indicates that after the end of work functional S T and T changes may be added to those of organic origin In the majority of individuals with coronary disease the S T and T changes during work and in the first few minutes afterwards are probably conditioned by the ischemic process This is supported by the observation of Kaijser (12) that there is a good correlation between the intensity of work and the degree of S T and T changes during as well as after work in patients with coronary insufficiency In the present study the S T changes at the end of work and three minutes after work were identical in all patients with clinically established coronary insufficiency This tends to refute the presence of an additional component (i e a varying degree of *autonomic tonus) behind the ST T changes after* work

It is more likely that the reduction elicited by propranolol in the S T and T changes after the end of work was due to the ventricular load and hence to the degree of ischemia being less pronounced in the work test after propranolol Thus in studies of how beta adrenergic receptor blocking agents influence the central circulatory adaption during work it has been shown that cardiac output left ventricular work and ventricular ejection rate during work are all significantly lower after the administration of propranolol than before (5 6 8 15 19) In view of this the present patients were allowed—as far as their subjective experience of the work permitted—to perform a somewhat larger amount of work after the propranolol blockade than before Even so there was no case in

which the heart rate after propranolol rose as high as it had during the first work test It is thus conceivable that in spite of the greater amount of work performed the strain on the myocardium and hence the degree of ischemia were not as pronounced during the second work test as during the first This is also suggested by the observation that the recovery from work as judged from the heart rate was somewhat more rapid after propranolol which has also been reported by Cumming and Carr (5)

In the group of patients with questionable coronary heart disease the electrocardiographic changes were reduced after propranolol not only after the end of work but also during work It is possible that in cases with only a moderate deterioration in the coronary circulation the reduction of left ventricular work and hence the oxygen requirement of the myocardium induced by propranolol results in an improved electrocardiographic picture even during exercise whereas in more advanced cases this effect does not materialize until after the end of work However the group of patients with questionable coronary heart disease included those with relatively unspecific symptoms and consequently the electrocardiographic changes may have been partly of a functional nature The etiological background to these observations can only be elucidated by further ols

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REFERENCES

1 Astrand I Exercise electrocardiograms recorded twice with an 8 year interval in a group of 204 women and men 48–63 years old Acta med scand 178 27 1965

2 Biorck G Ergotamine and apparent coronary insufficiency Brit Heart J 9 181 1947

3 Blackburn H Keys A Simonson E Rautaharju P & Punsar S The electrocardiogram in population studies A classification system Circulation 21 1160 1960

4 Blomquist G The frank lead exercise electrocardiogram A quantitative study based on averaging technic and digital computer analysis Acta med scand Suppl 440 1965

5 Cumming G R & Carr W Hemodynamic response to exercise after propranolol in normal subjects Canad J Physiol Pharmacol 44 465 1966

6 Epstein S E Robinson B F Kahler R L & Braunwald E Effects of β adrenergic blockade on the cardiac response to maximal and submaximal exercise in man J clin Invest 44 1745 1965

7 Furberg C Adrenergic beta blockade and electrocardiographical ST T changes Acta med scand 181 21 1967

8 Hamer J & Sowton E Cardiac output after beta adrenergic blockade in ischaemic heart disease Brit Heart J 27 892 1965

9 Holmgren A & Mattson K H A new ergometer with constant work load at varying pedalling rate Scand J clin Lab Invest 6 137 1954

10 Holmgren A Jonsson B Levander M Linderholm H Sjostrand T & Strom G ECG changes in vasoregulatory asthenia and the effect of physical training Acta med scand 165 259 1959

11 Holmgren A & Strandell T On the use of chest head leads for recording of electrocardiograms during exercise Acta med scand 169 57 1961

12 Kaijser L EKG forandringar vid koronarinsufficiens som funktion av arbetsintensitet och duration Lakartidn 63 3340 1966

13 Kirchhoff H W Zur Wirkung einer β adrenolytischen Substanz in Belastungs und Kipptisch Untersuchungen Z Kreisl Forsch 55 583 1966

14 Lepeschkin E W Über das Electrocardiogramm bei experimenteller Koronar Insuffizienz Cardiologia (Basel) 2 236 1938

15 Mahon W A The hemodynamic effects of beta adrenergic blockade in man Clin Res 13 213 1965

16 Mattingly T W The postexercise electrocardiogram Its value in the diagnosis and prognosis of coronary arterial disease Amer J Cardiol 9 395 1962

17 Nordenfelt O Über funktionelle Veranderungen der P und T Zachen im Electrocardiogramm Acta med scand Suppl 119 1941

18 — Orthostatic ECG changes and the adrenergic beta receptor blocking agent propranolol (Inderal) Acta med scand 178 393 1965

19 Paley H W McDonald I G & Peters F W Effect of beta adrenergic receptor suppression on left ventricular response to exercise in man Circulation Suppl ? 167 1965

20 Robb G P Marks H H & Mattingly T W The value of the double standard two step exercise test in the detection of coronary disease Trans Ass Life Insur med Dir Amer 40 52 1957

21 Scherf D & Schlachman M Electrocardiographic and clinical studies on the action of ergotamine tartrate and dihydroergotamine Amer J med Sci 216 673 1948

22 Strait G B & Bruce R A Nonspecific and beta adrener ic blocking effects of Alderlin in angina pectoris Amer Heart J 70 150 1965

23 Suzman M M Diagnostic significance of posture hyperventilation exercise and beta adrenergic blockade in the differentiation of anxiety induced from ischemic cardiac disorders Circulation Suppl 3 225 1966

24 Wood P McGregor M Magidson O & Whitaker W The effort test in angina pectoris Brit Heart J 1? 363 1950

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CAPILLARY PERMEABILITY SURFACE AREA PRODUCT (*PS*) OF RENKIN IN HUMAN SKELETAL MUSCLE

Effect of Locally Applied Norepinephrine

PRELIMINARY REPORT

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The transcapillary exchange of water soluble substances is dependent upon the permeability of the capillary wall to the substance in question (*P*) and the surface area of the capillary bed open to flow (*S*) (4) Transcapillary exchange expressed as the clearance of a substance (C_i in ml whole blood/min/100 g tissue) from blood to tissue or vice versa is a function of blood flow (Q in ml whole blood/min/100 g) and *PS* (ml/min/100 g) the permeability surface area product or capillary diffusion capacity introduced by Renkin (7) which is the flux across the capillary surface per 100 g tissue at unit concentration difference (with concentrations referred to whole blood) Thus

$$C_i/Q = 1 - \exp(-PS/Q) \qquad (1)$$

In contrast to water soluble substances the transcapillary exchange of small lipid soluble substances (e g xenon) is limited only by blood flow This clearance thus measures capillary blood flow at all flow rates (5) Clearance is also the product of the clearance constant k and the partition coefficient between blood and tissue λ Thus

$$C_i = k_i \ \lambda_i \ 100 \qquad (2)$$

where C_i is expressed in ml/min/100 g Combining equations 1 and 2 and assuming that $Q = C_X$ (clearance of xenon) yields

$$\frac{k_i}{k_X} = \frac{\lambda_X}{\lambda_i}\left[1 - \exp\left(-\frac{PS}{k_X \ \lambda_X \ 100}\right)\right] \qquad (3)$$

A detailed derivation of formula 3 with special reference to transport from tissue to blood is given elsewhere (3) We have applied this formula to the calculation of *PS* in human skeletal muscle at rest by measuring the simultaneous tissue clearance of ^{24}Na and ^{133}Xe (5) λ_X is 0 7 for skeletal muscle at normal hematocrit (2) The sodium space of skeletal muscle is 13 % (1) Assuming that Na is extracellular and has approximately equal concentrations in interstitial space and plasma λ_{Na} at normal hematocrit is about 0 24 With these data it is then possible to calculate *PS* k_{Na}/k_{Xe} approaches λ_X/λ_{Na} as Q (or k_X) approaches zero Error in the assumed value of λ_X/λ_{Na} changes the y intercept of the *PS* curves (see Fig 1) and the absolute values of *PS* but changes neither their general form nor the physiological conclusions

Lassen (6) has recently applied an approximate formula to the calculation of maximal diffusion capacity in exercising muscle which differs from *PS* by (1 Hematocrit) and by the approximation of a fairly low extraction from tissue to blood at high flows

PS in resting muscle of healthy adult volunteers was 1 52 ± 0 46 ml/min/100 g (35 injections in 13 subjects) (Fig 1) With 0 04 mg/ml norepinephrine locally (42 injections in 13 subjects) *PS* was 0 46 ± 0 20 ($P < 0$ 001) Note the change in the shape of the *PS* curve illustrating that equal capillary blood flow does not necessarily mean equal transcapillary exchange

The analytical procedure described here affords an opportunity for relatively easily obtaining data of fundamental physiological importance concerning the capillary bed in human skeletal muscle Characterization of the transport function of the capillary bed in clinical situations not only at maximum transport rates but also with a capillary bed of normal or less than normal

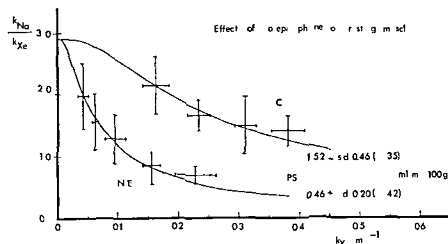


Fig 1 k $/k_X$ vs k_X as classed mean values following simultaneous intramuscular injection of Na and ^{133}Xe in human tibialis *anterior muscle C = at rest N.E* = at rest with addition of norepinephrine The solid lines were calculated from formula 3 with the mean *PS* of the 2 groups

capacity is stressed We are primarily interested in changes of *PS* at subphysiological flows such as can be seen in shock hemorrhage and trauma Such studies are now in progress both in experimental shock models and in severely ill patients

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REFERENCES

Agnew W F Effects of plasma tonicity on the distribution of water and solutes in brain and muscle Exp Neurol 13 58 1965

2 Andersen A M & Ladefoged J Partition coefficient of ^{133}xenon between various tissues and blood in vivo Scand J clin Lab Invest 19 72 1967

3 Appelgren L & Lewis D H In preparation

4 Landis E M & Pappenheimer J R Exchange of substances through the capillary walls Handbook of physiology Section 2 Circulation Vol II p 961 Williams & Wilkins Washington 1963

5 Lassen N A Muscle blood flow in normal man and in patients with intermittent claudication evaluated by simultaneous ^{133}Xe and Na clearances J Clin Invest 43 1805 1964

6 — Capillary diffusion capacity of sodium studied by clearances of Na and ^{133}Xe from hyperemic skeletal muscle in man Scand J clin Lab Invest Suppl 99 24 1967

7 Renkin E M Transport of K from blood to tissue in isolated mammalian skeletal muscle Amer J Physiol 197 1 05 1959

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GLUCOSE TOLERANCE PLASMA LIPIDS AND SERUM INSULIN IN PATIENTS WITH ISCHAEMIC HEART DISEASES

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Abst act The glucose tolerance the insulin concentration in serum and concentrations of free fatty acids (FFA) triglyceride and cholesterol in plasma or serum were examined in 25 patients of normal weight and below the age of 65 years they were all suffering from ischaemic heart diseases (angina pectoris and/or earlier episodes of coronary occlusion) A clinical diagnosis of diabetes mellitus was not established in any of these cases the patients were not predisposed to diabetes and neither hypertension nor reduced renal function was observed in any of the pat ents Patients who on an earl er occasion had experienced episodes of coronary occlusion were seen *at least three years after such occurrence*

Among the 25 patients the glucose tolerance was reduced in 12 ($k < 1\ 10$) determinations being made by glucose elimination after intravenous appl cation of glucose In patients in whom the glucose tolerance was found to be reduced concentrations of insul n were reduced and FFA elevated suggesting latent diabetes mellitus In pat ents in whom the glucose tolerance remained normal the insulin concentrat on in serum was found to be abnormally high

Whether the demonstrated abnormal metabol sm may be of signif cance for the pathogenesis of ischaemic cardiovascular les ons rema ns to be defined

The increased incidence of ischaemic heart diseases in patients with diabetes mellitus has been ascertained beyond doubt on the basis of autopsy findings and clinical observations (9 10 11 17 18 19 20 36 41) Furthermore in recent investigations the glucose tolerance has been found to be reduced in up to 50 per cent of patients with ischaemic heart lesions in whom clinically recognizable symptoms of diabetes were absent (1 4 13 15 24 26 30 31 34 35 39 40 42) It is generally admitted however that glucose tolerance may be influenced by a number of factors Consequently the present study was designed with a view to 1 defining whether a reduced glucose tolerance in patients with ischaemic heart lesions might be demonstrable also in individuals in whom factors known to affect the glucose tolerance were absent such as hereditary predisposition old age (6 23) obesity (43) hypertension (5 25 44) reduced renal function (37) medication by diuretics or steroids and recovery from earlier episodes of coronary occlusion (33 34) and to 2 examining whether such decreased glucose tolerance coincides with changes in concentrations of insulin and lipids in serum analogously with changes seen in cases of frank diabetes mellitus

MATERIAL

The material comprises 25 patients (2 women 23 men) w thout clinically recognizable d abetes who at the time of examination were suffering from ischaemic heart le sions In nine of the pat ents characteristic angina pectoris in relation to effort was manifest coronary occlusion had occurred in the remain ng 16 patients at least three years before the present examination. The d agnoses were in all cases verified by characteristic changes seen by electrocardiography and the elevated concentrations of enzymes in serum At the t me of examination the patients concerned were not hospitalized they were not even conf ned to bed All patients rece ved treatment by anticoagulants but otherwise they had no med cal or d etetic treatment The following criteria were fulfilled in all cases age below 65 years we ght within the range ± 10 per cent Danish average weight (Hafnia (Danish Life Insurance Company) statistics 1958) arterial blood pressure below 170 105 mm Hg serum-creatinine concentration below 1 10 mg/100 ml absence of congestive heart failure There was no family history of diabetes n any of the cases

METHODS

Intra enous glu ose tolerance tests according to Lundbæk s method (1) and electrocard ography were carried out in all cases The glucose concentration in capillary blood was determined accord ng to Hagedorn s and Nor

Table I *I v glucose tolerance test fasting blood sugar triglycerides and cholesterol in patients with ischaemic heart disease*

No	Age	Sex	Cardiac pain	Coron occl	Per cent of Danish mean body weight (Hafnia)	K value (Normal ≥ 1 10)	Fasting blood-sugar (mg/100 ml) (Normal = 80–120)	Triglycerides (mmol/l) (Normal = 0 46–1 98)	Cholesterol (mg/100 ml) (Normal = 170–240)
Cases with reduced glucose tolerance									
1	56	♂	+	0	99	0 41	127	1 69	275
2	52	♂	+	+	110	0 77	80	1 91	260
3	58	♂	+	0	106	0 83	114	0 48	259
4	61	♂	+	0	94	0 85	108	0 59	292
5	49	♂	+	+	87	0 87	116	1 33	197
6	48	♂	+	0	110	0 90	98	1 16	232
7	57	♂	+	+	101	0 91	104	0 42	301
8	60	♂	+	+	86	0 92	69	0 14	250
9	62	♂	+	0	104	0 97	106	1 50	285
10	41	♂	0	+	107	1 00	91	1 16	355
11	60	♀	+	0	89	1 03	97	0 97	320
12	58	♂	+	0	86	1 08	63	0 67	212
Mean	55				98 3	0 879	97 8	1 00?	269 9

man Jensen s method (1_) at intervals of 10 20 30 40 50 60 70 80 90 and 1 0 min after intravenous application of 25 g of d glucose Concentrations of insulin in serum and of free fatty acids (FFA) in plasma were determined prior to application of glucose and subsequently at intervals of 10 30 60 and 120 min after the application Concentrations of total cholesterol and triglyceride in serum were determined while the patients were in the fa ting state

The insulin concentration was determined immunologically in heparinized serum using the double antibody technique suggested by Hales & Randle (7) (¹²⁵I labelled insulin from the Niels Steensen Hospital) FFA was determined according to Dole s method (8) the triglyceride concentration according to Laurell s method (16) the cholesterol concentration being determined according to Libermann-Burchardt s method (?7) All analyses were carried out in duplicate The analytical reproducibility given as the coefficient of variation (100 s/m) was as follows glucose concentration _9 insulin concentration 9 4 FFA 5 0 % triglyceride concentration 3 0% and cholesterol concentration 3 6

Table II *I v glucose tolerance test fasting blood sugar triglycerides and cholesterol in patients with ischaemic heart disease*

No	Age	Sex	Cardiac pain	Coron occl	Per cent of Danish mean body weight (Hafnia)	K value (Normal ≥ 1 10)	Fasting blood sugar (mg/100 ml) (Normal = 80–120)	Triglycerides (mmol/l) (Normal = 0 46–1 98)	Cholesterol (mg/100 ml) (Normal = 170–240)
Cases with normal glucose tolerance									
13	57	♂	+	0	94	1 11	98	1 69	259
14	61	♂	+	0	102	1 22	103	0 77	284
15	60	♂	0	+	94	1 27	87	0 84	287
16	53	♂	0	+	100	1 36	105	1 39	288
17	56	♂	+	+	108	1 44	60	2 23	221
18	60	♀	+	+	105	1 49	105	0 90	356
19	53	♂	+	+	93	1 51	75	1 09	4 1
20	40	♂	0	+	97	1 58	111	0 59	263
21	60	♂	0	+	105	1 59	87	1 61	377
22	43	♂	0	+	86	1 67	87	0 64	235
23	49	♂	0	+	86	1 87	89	1 44	346
24	60	♂	0	+	93	1 93	133	0 58	316
25	56	♂	+	+	94	2 01	86	2 96	374
Mean	54 6				96 7	1 542	94 3	1 288	309 8

The glucose tolerance was estimated on the basis of the slope (K) of the fall in the blood sugar concentration in a semi logarithmic system where $K = 100 \log 2/t_{\frac{1}{2}}$ The figure $t_{\frac{1}{2}}$ denotes the time in minutes until the glucose concentration is reduced by 50

According to Wahlberg (47) *normal* values of glucose tolerance have been found to be $K \geq 1\ 10$ In our laboratory the following normal values were observed fasting insulin concentration 15– 9 μU/ml (14) fasting FFA ?70–934 mEq/l fasting cholesterol concentration 170– 40 mg/100 ml and fasting triglyceride concentration 0 46–1 98 mmol/l

RESULTS

The findings appear from Tables I–III and from Figs 1–3 Among the 25 patients the glucose tolerance was found to be reduced in 12 ($K <$ 1 10) *An elevated fasting glucose concentration* was observed in one of these patients and in one *in whom the glucose tolerance remained normal*

Elevated fasting insulin concentrations in serum *were seen in eight patients (Tables I and II)* in six of the latter the glucose tolerance remained *normal Fasting insulin concentrations in serum* were not found to be reduced in any of the *patients*

Mean concentrations of insulin following in*jection of glucose were slightly higher in patients* than in normal subjects (Fig 1) In patients with *normal glucose tolerance the concentration of in*sulin was higher than concentrations observed in *patients with reduced glucose tolerance (Fig 2* and Table III) and higher than those observed in *normal subjects examined 10 30 and 60 min after* injection of glucose (Table III) In patients pre*senting reduced glucose tolerance the insulin con*centration in serum was found to be significantly *below normal level 10 min after injection of* glucose and significantly higher 60 min after the *injection (Fig 2 and Table III)*

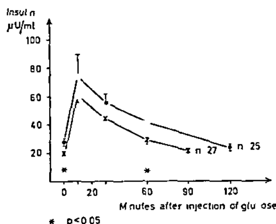


Fig 1 Insulin concentration (mean ± S.E.M.) prior to and during intravenous glucose tolerance tests in young normal subjects (×——×) and in patients with ischaemic heart diseases (●——●)

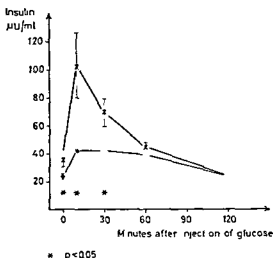


Fig 2 Insulin concentration (mean ± S.E.M.) prior to and during glucose tolerance tests (GTT) in patients with ischaemic heart diseases ×——× patients with normal GTT (n = 13) ●——● patients with reduced GTT (n = 12)

Table III *Insulin concentration (mean ± standard deviation) before and during i v glucose tolerance test in young healthy people and in patients with ischaemic heart disease*

	No	Fasting	10	30	60	90	1 0
A	7	19 6 ± 5 1	60 7 ± 22 0[a]	44 0 ± 12 0	9 2 ± 9 9	21 6 ± 5 7	
B	13	31 5 ± 13 3[b]	10? 0 ± 84 0[b]	69 2 ± 39 0[b]	44 5 ± 14 5[b]		23 7 ± 8 5
C	1.	23 7 ± 5 5[b]	4 2 ± 12 6[b]	41 8 ± 10 8[b]	38 8 ± 10 8[b]		24 1 ± 7 2

[a] p for A versus C < 0 05
[b] p for A versus B and B versus C < 0 05

A = normal healthy young people B = patients with normal glucose tolerance test C patients with reduced glucose tolerance test

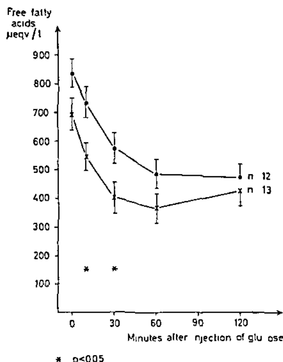


Fig 3 Free fatty acids (mean ±S E M) prior to and during glucose tolerance tests (GTT) in patients with ischaemic heart diseases ×——× patients with normal GTT (*n*=13) ●——● patients with reduced GTT (*n*=12)

Elevated fasting FFA values were observed in five of the patients in one of these the glucose tolerance remained normal (Tables I and II) The fall in FFA values following glucose injection was less marked in patients with reduced glucose tolerance than in patients with normal tolerance the FFA values obtained 10 and 30 min after injection of glucose being significantly different (Fig 3)

In 20 of the patients the fasting cholesterol concentration was elevated (Tables I and II) among these the glucose tolerance remained normal in 11 cases In two patients presenting normal glucose tolerance fasting triglyceride concentrations were elevated Concentrations of cholesterol and of triglyceride were found to range at the same level in the two groups of patients with normal and reduced glucose tolerance respectively

Only four out of the 25 patients presented normal concentrations of the three lipides examined the glucose tolerance was reduced in three of these patients

A correlation between electrocardiographic changes subjective symptoms and glucose tolerance was not demonstrable

DISCUSSION

According to a report by Wahlberg (42) concerning a control material comprising patients without hereditary predisposition to diabetes and without hypertension obesity or ischaemic heart diseases the glucose tolerance was found to be reduced in 8 % of all individuals in the age group 32 to 89 years

As regards selection and composition the material here discussed conforms closely to the one reported by Wahlberg except that patients in the former group were suffering from ischaemic heart diseases *In the present material the incidence* of reduced glucose tolerance was however six tims as high as that observed by Wahlberg Similar reduction of the glucose tolerance has been observed by several other authors in patients with ischaemic heart diseases (1 4 24 26 30 31 39 42)

The reduced glucose tolerance can hardly be explained as a result of physical inactivity since all patients concerned had been up and about at the time of examination It cannot be explained either as a result of the stress condition following coronary occlusion because the interval between the occurrence of the latter and the present examination was three years Consequently the sign must presumably be co existent with the ischaemic heart disease of the patients concerned

In fact the patients seem to form two groups the glucose tolerance remaining normal in one group and being reduced in the other

A characteristic of patients presenting reduced glucose tolerance was the less marked and late occurring rise in the insulin concentration in serum together with the relatively high FFA concentration following application of glucose These features correspond closely to those seen in patients with maturity onset diabetes (22 32)

The abnormally high insulin concentration in serum in patients with normal glucose tolerance whether in the fasting state or following glucose injections conforms with findings by Peters and Hales (28) and Nikkila et al (24) who studied patients with angina pectoris and patients with recent coronary infarction According to these investigators and also to findings in the present material a normal glucose tolerance is maintainable exclusively if the organism manages to raise the insulin concentration in serum Chlouverakis et al (3) observed that normal glucose tolerance

could not be maintained unless the insulin concentration in serum rose in parallel with increasing age Whether the presence of insulin antagonists (38) or inhibition of the glucose transport owing to the vascular lesion is responsible remains to be defined

The absence of a correlation between cholesterol or triglyceride concentrations in serum and glucose tolerance is in accordance with findings reported by Carlson and Wahlberg (2)

Thus the previously observed co-existence of ischaemic heart lesions and reduced glucose tolerance has been found in the present study to be valid also in cases of non hypertensive patients of normal weight in whom the renal function remains normal patients who have never experienced episodes of heart failure and who do not receive diuretic drugs In addition the reduced glucose tolerance was found to be accompanied by changes of the insulin concentration in serum and of the FFA concentrations thus suggesting the presence of latent diabetes mellitus

According to Sivers et al (33) and Wahlberg (42) the prognosis in cases of ischaemic heart disease seemed to be less favourable in patients with reduced glucose tolerance than in those in whom the tolerance remained normal It might be of value to study whether the prognosis in cases of latent diabetes mellitus might become more favourable if the glucose tolerance were normalized by daily medication with for instance tolbutamide

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REFERENCES

1 *Braunsteiner H* d Pauli R Sailer S & Sandhofer F Klin Wschr 43 585 1965
2 Carlson L A & Wahlberg F Acta med scand 180 307 1966
3 Chlouverakis C Jarrett R J & Keen H Lancet 1 806 1967
4 Cohen A M & Shafir E Diabetes 14 84 1965
5 Conn J W New Engl J Med. 273 1135 1965
6 Crockford P M Harbeck R J & Williams R H Lancet 1 465 1966
7 Deckert T & Hagerup L. Acta med. scand 18_ 225 1967
8 Dole V P J clin Invest 35 1950 1956
9 Eckerstrom S Acta med scand Suppl 50 139 1951
10 Feldman M & Feldman M Jr Amer J med Sci 2?8 53 1954
11 Goldenberg S Alex M & Blumenthal H T Diabetes 7 98 1958
1? Hagedorn H C Halstrøm F & Norman Jensen B Rep Steno Hosp (Kbh) 1 _9 1946
13 Hagerup L & Deckert T Unpublished results
14 Herman M V & Gorlin R Amer J Med 38 481 1965
15 Keen H Rose G Pyke D A Boyns D Chlouverakis C & Mistry S Lancet 1 505 1965
16 Laurell S Scand J clin Lab Invest 18 668 1966
17 Levine S A & Brown C L Medicine (Baltimore) 143 _45 19 9
18 Liebow J M Hellerstein, H K & Miller M Amer J Med 18 438 1955
19 Linden L Acta med scand 143 464 195
20 Lisa J R Magidav M Galloway I & Hart J F J Amer med Ass 1 0 19 1942
21 Lundbæk K Brit med J 1 1507 196
?2 Melani F Lawecki J Bartelt, K M & Pfeiffer E F Diabetologia ? 210 1966
3 Nilsson S E Acta med scand Suppl 4 8 1964
4 Nikkila E A Miettinen T A Vesenne M R & Pelkonen R. Lancet ?08 1965
25 Nye E R Brit med J _ 727 1964
6 Ostrander L D Francis T Hayner N S Kjelsberg M O & Epstein F H Ann intern Med 6? *1188 1965*
27 Pearson S Stern S & McGavack, T H J clin Endocr 12 1245 195?
_8 Peters N & Hales C N Lancet 1 1144 1965
?9 Rapaport C M & Hurd H F Arch intern Med *113 405 1964*
30 Reaver G Calciano A Codv R Lucas C & Miller R J clin Endocr 23 1013 1963
31 Schrade W Boehle E & Biegler R Lancet 1409 1960
3 Seltzer H S Allen E W Herron, A L & Brennan M T J clin Invest 46 3?3 1967
33 Sievers, J Blomqvist G & Bjork G Acta med scand 169 95 1961
34 Soloff L A & Schwartz, H Lancet 1 449 1966
35 Sowton W Brit med J 1 85 196?
36 Stearns, S Schlesinger M J & Rudy A Arch intern. Med 80 463 1947
37 Tchobroutsky G Collin de L Hortet G Rosselin G Assan R & Derot M Diabetologia 1 101 *1965*
38 Vallance-Owen J & Ashton W L Lancet 1 1? 6 1963
39 Wadell, W R & Feld R. A Metabolism 9 800 *1960*
40 Wahlberg, F Acta med scand 171 1 196
41 — Amer Heart J 65 749 1963
4 — Acta med scand Suppl 453 1966
43 Walker J B Ciba Found Colloq on Endocrinol 15 5 1964
44 Welborn T A Breckenridge A Rubinstein A H Dollery C T & Fraser T R Lancet 1 1336 1966

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VOLUME CHANGES OF THE CALF DURING TEN MINUTES VENOUS STASIS

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Abstract Volume changes were examined in ten normal young experimental subjects by measurements on the calf with an air filled rubber cuff plethysmograph during venous stasis Part of the experiment was performed while breathing 10 oxygen in nitrogen The interpretation of the volume changes is discussed and it is suggested that from the fourth minute they are an expression of capillary filtration rate After the initial rise which is due to arterial blood flow there is a transition phase where the volume increase is due to both venous filling and capillary filtration. Ten minutes breathing of 10 oxygen in nitrogen gave no changes in capillary filtration rate

The capillary filtration rate (CFR) in the extremities can be measured by plethysmography Krogh et al (8) for example have used this method to determine the CFR in man The principle of the measurement is that the increase in volume of an extremity or of a part of an extremity is measured during venous stasis the assumption being that when the initial increase in volume ceases the elevated venous pressure during the stasis will cause an increased capillary pressure resulting in a rise in the filtration from the capillaries

The present study aims at a closer analysis of filtration processes in the calf of the leg as well as at a description of the hemodynamic conditions which determine the processes of filtration in the extremities

METHOD

An air filled rubber cuff plethysmograph (4) was placed on the thickest part of the calf Venous occlusion was produced by means of a blood pressure cuff just proximal to the patella The changes in volume were evaluated by changes in pressure in the plethysmograph recorded with a capacitance manometer The signal was amplified and passed to a linear recorder

The effect of venous occlusion lasting for 10 min was recorded

Part of the experiment was performed while breathing 10% oxygen in nitrogen from a Douglas bag which was continuously filled with this gas mixture from a gas tank

Experimental procedure

After recumbent rest for 30 min, the experimental subjects were examined on their left calf by the plethysmograph The venous occlusion was produced on the thigh by pressures of 40 60 and 70 mm Hg for periods of 10 min while breathing atmospheric air with at least 5 min pauses between the different pressures The recording was repeated at a venous occlusion pressure of 60 mm Hg while breathing 10 oxygen in nitrogen The experimental subjects had been breathing the gas mixture for 10 min before the measurement

The room temperature varied between ~ 6 C but was constant during the individual measurement

MATERIAL

The studies were performed with ten experimental subjects (6 men and 4 women) with a mean age of 23 years (from 0 years to 31 years)

RESULTS

Fig 1 shows a plethysmograph curve during and after a 10 minute period of stasis The figure is drawn on the basis of the mean values from the experiments at 60 mm Hg while breathing atmospheric air It is seen that the curve contains three different phases The initial slope in the plethysmograph curve expresses the resting blood flow The second phase of the curve is seen in the 2nd minute (II) and the third goes from the 4th to the 10th minute On releasing the occlusive pressure an immediate decrease in the volume is observed followed by a horizontal level for 5–10 seconds The volume then slowly decreases The final level not shown in the figure is reached after 2–3 min

Table I shows the mean values of the plethys

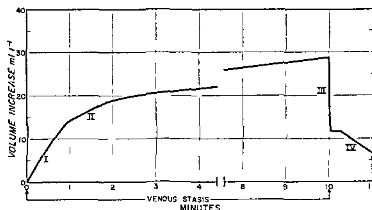


Fig 1 Plethysmograph curve during and after a 10 minute period of stasis

mographic measurements while breathing atmospheric air The resting blood flow/volume was very similar (15 7 14 3 and 17 2 ml min⁻¹ l⁻¹) at 40 60 and 70 mm Hg venous stasis All other values in the table increase with increasing cuff pressure

Table II shows the results of plethysmography with a venous stasis pressure of 60 mm Hg while breathing 10% oxygen in nitrogen and atmospheric air respectively As a result of the reduction in the oxygen content in the inspired air there is a significant rise in resting blood flow/volume of 6 5 ml min^{-1} l^{-1} ($0\ 005 > P$)

There is also a significant rise in the volume increase in the 2nd minute of 1 8 ml min^{-1} l^{-1} ($0\ 01 > P$) The change in the volume increase from the 4th to the 10th minute is not significant ($P > 0\ 05$)

The values for the immediate decrease in volume and the subsequent gradual decrease in volume after release of stasis (III and IV in Fig 1) were calculated but are not presented in the tables The

Table I *Mean values of the Plethysmographic measurements while breathing atmospheric air*

Cuff pressure (mm Hg)		Resting blood flow/volume (ml min⁻¹ l⁻¹)	Volume increase in the 2nd min (ml min⁻¹ l⁻¹)	Capillary filtration rate (ml min⁻¹ l⁻¹)
40	$\bar{x}$	15 7	3 4	0 63
$n=10$	SD	9 4	2 2	0 22
60	$\bar{x}$	14 3	5 0	1 12
$n=10$	SD	6 7	1 8	0 34
70	$\bar{x}$	17 2	6 7	1 37
$n=10$	SD	7 2	2 4	0 53

Table II *Mean values of the plethysmographic measurement with a venous stasis pressure of 60 mm Hg and breathing respectively atmospheric air and 10 % oxygen in nitrogen*

Cuff pressure (60 mm Hg)		Resting blood flow/volume (ml min⁻¹ l⁻¹)	Volume increase in the 2nd min (ml min⁻¹ l⁻¹)	Capillary filtration rate (ml min⁻¹ l⁻¹)
Atmospheric air	$\bar{x}$	14 3	5 0	1 12
$n=10$	SD	6 7	1 8	0 34
10 % oxygen in nitrogen	$\bar{x}$	20 8	6 8	0 90
$n=10$	SD	9 1	3 2	0 50
Difference		+6 5	+1 8	−0 22
P		$0\ 005 > P > 0\ 001$	$0\ 01 > P > 0\ 005$	$0\ 10 > P > 0\ 05$

results showed that total volume increase immediate volume decrease and slow volume decrease all increased with increasing cuff pressure There was no significant difference in these values as a result of breathing 10 % oxygen

DISCUSSION

When venous pressure is applied to an extremity a linear increase in volume will be observed during the first 10–20 seconds as an expression of the arterial blood flow as the venous return is stopped while the arterial flow continues The pressure on the venous side will rise gradually and venous return under the occlusion cuff becomes reestablished The question is when this state is reached in the human calf

Frey (5) showed that venous return starts after 20 seconds in the hand By simultaneous plethysmographic measurement on the hand and determination of venous pressure distal to the stasis cuff Frey showed that venous pressure rises to a constant value in 20 seconds while the volume increase continues although at a reduced rate It has further been shown that an increase in femoral venous pressure of 10 mm Hg in the isolated dog hind limb resulted in stabilized peripheral venous pressure (digital vein) in less than 30 seconds (3) These studies included measurements of femoral venous pressure peripheral venous pressure in a digital vein arterial pressure leg weight and venous outflow in the isolated dog hind limb Following elevation of outflow pressure there was an immediate increase in all pressures and a rapid *increase in weight* (volume) associated with a pronounced transient decrease in venous outflow below the constant arterial inflow These abrupt changes were followed by a leveling off of all pressures at values above control (the magnitude of change being dependent on the increment in the outflow pressure) and a slow steady gain in limb weight (volume = capillary filtration) which was associated with a venous outflow value slightly below the value for arterial inflow When venous outflow pressure was lowered exactly analogous changes occurred in the opposite direction

As shown in Fig 1 a volume curve containing three different phases is obtained by plethysmographic measurement on the calf during venous stasis After three minutes a slow continuous rise is seen However when recording more distally on the extremity volume curves containing only two different phases were obtained the second phase starting after the first minute These types of curves were obtained by Celander (2) on the foot of newborn infants and by Nagasaka (9) on the fingers of adults These differences are explained by the different contribution of skin and muscle at the various levels of the extremities

The slope of the curve during the 2nd minute (II in Fig 1) which indicates a volume increase could be explained by a gradual dilatation of the veins or in other words a pooling of the blood on the venous side exclusively indicating that the venous pressure during the 2nd minute has not reached the tissue pressure caused by the stasis cuff However a venous outflow exists already after one minute This may be demonstrated by further raising the venous occlusion pressure during a period of stasis Thus it is indirectly shown that venous pressure after one minute is equal to the tissue pressure obtained from the stasis cuff Owing to the valves in the communicating veins it is possible to have different pressures in the superficial and profound venous system Measuring the venous pressure distally in a superficial vein would only give information on the pressure in the veins between the proximal and distal valves The pressure in the profound venous system might be higher

The slope in the 2nd minute in Fig 1 therefore expresses venous filling especially of the superficial venous system although capillary filtration must contribute On account of the valves in *the communicating veins an outflow from the* profound venous system may well exist while this is not possible for the superficial venous system

As a result of the filtration in the muscle the pressure in the muscle tissue rises so that there is a counterpressure to the capillary pressure resulting from the venous stasis Filtration will therefore again decrease as shown by Jacobsen et al (7) and when there is no longer possibility of further increase in volume within the muscle fascia a steady state develops between fluid transport from the capillaries to the tissues and transport back from the tissues to the capillaries The present investigation does not elucidate when this equilibrium is reached

It may be assumed that the cutaneous filtration does not meet with any essential tissue

counterpressure during the period of measurement The cutaneous filtration will therefore continue unimpeded This is in accordance with clinical experience that edema of the skin may be monstrous while edema of the muscle is always moderate

Consequently the volume increase during the 2nd minute cannot be caused by capillary filtration alone as suggested in a preliminary report (10) In previous studies (10) it was shown that moderate acute carbon monoxide exposure significantly augmented the volume increase during the 2nd minute Further experiments have confirmed this observation Breathing 100% oxygen or 15% oxygen in nitrogen did not change the slope while breathing 10% oxygen in nitrogen significantly increased the slope in phase II as is also shown in Table II It is impossible at present to explain this observation

Asmussen et al (1) applied an air filled plethysmograph on the calf and used the volume increase from the 2nd to the 7th minute as an expression of capillary filtration In the present work the volume increase from the 4th minute has been used to indicate capillary filtration

Experimental confirmation of the theoretical considerations for using a plethysmograph to measure capillary filtration rate has been established for example by Gayton et al (6) They measured fluid movement through the capillary membrane in the lower legs of dogs by using implanted perforated capsules as internal plethysmographs By this technique it was possible to compare a decrease in the interstitial fluid pressure with the effects of changes in venous pressure and arterial pressure Their results confirmed Starling s hypothesis that changes in interstitial fluid pressure can affect movement of fluid through the capillary membrane in the same way as do changes in capillary pressure plasma colloid osmotic pressure and interstitial fluid colloid osmotic pressure

If the stasis is released after the lapse of ten minutes the volume increase will be reduced at two different rates a rapid phase which must be due to the displacement of the intravascular volume and a slow phase determined by back diffusion of the fluid to the capillaries and possibly also transport through the lymphatic vessels The horizontal level of about 10 seconds duration at the transition between the two phases suggests that a certain time is taken to refill the capillary network which has become emptied as a result of the increased tissue pressure

Table II shows that exposure to 10% oxygen in nitrogen gave no increase in capillary filtration rate in the leg An increase was to be anticipated since high altitude experiments (11) have shown a hemoconcentration and a decrease in plasma volume indicating an increased capillary filtration rate However the present experiment only exposed the subjects to 10% oxygen in nitrogen for ten minutes before the measurements This exposure seems to be of too short duration to provoke any effect on the capillary filtration

In calculating the capillary filtration coefficient (CFR/mm Hg) Celander (2) assumes that a rise in the venous stasis pressure causes a rise of 80% in capillary pressure For this reason the difference between volume increases at 60 mm and 40 mm Hg stasis pressure has been divided by 16 instead of 20 in order to obtain an expression of volume increase per mm Hg Volume increase from the fourth to the tenth minute per mm Hg thus becomes 0 031 ml min^{-1} l^{-1} mm Hg^{-1} This is of the same order of magnitude as the CFR/mm Hg obtained by other authors

ACKNOWLEDGEMENTS

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REFERENCES

1 Asmussen E & Knudsen E O E Acta physiol scand 6 67 1943
2 Celander O & Mårild K Acta paediat (Uppsala) 51 385 1962
3 Diana J N Colantio R & Haddy F J Amer J Physiol 212 456 1967
4 Dohn K Cravenhorst J S & Jarloev N V Rep Steno Hosp (Kbh) 6 141 1956
5 Frey H M M Scand J clin Lab Invest 19 346 1967
6 Guyton A C Prather J Scheel K & McGehee J Circulat Res 19 1022 1966
7 Jacobsen S & Kjellmer I Acta physiol scand 60 286 1964
8 Krogh A Landis E M & Turner A H J clin Invest 11 63 1932
9 Nagasaka T Jap J Physiol 15 423 1965
10 Siggaard Andersen J Kjeldsen K Petersen F B & Astrup P Acta med scand 187 397 1967
11 Siggaard Andersen J Petersen F B Hansen T & Mellemgaard K Scand J clin Lab Invest In print

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SERUM AND URINARY URIC ACID IN RESPIRATORY ACIDOSIS

PRELIMINARY REPORT

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Abstract Serum and urinary uric acid were measured in seven patients suffering from severe respiratory acidosis The highly elevated serum levels of uric acid decreased to normal after carbon dioxide had been blown off with assisted ventilation Simultaneously the urinary excretion of uric acid increased

The development of secondary hyperuricaemia may be due to increased uric acid formation or decreased elimination Elimination is principally achieved through excretion by the kidneys Although this mechanism is not fully understood increasing evidence supports the current view that uric acid after glomerular filtration is largely reabsorbed and later actively secreted in distal tubuli (17) Agents which increase tubular resorption or impair tubular secretion may cause hyperuricaemia

It has been shown that the infusion of lactic acid considerably decreases renal uric acid elimination in normal healthy men without a change in the Inulin or PAH clearances (18) In several other clinical conditions known to be associated with increased lactic acid levels the serum uric acid has been shown to be elevated in toxaemia of pregnancy (6) after physical effort (19) after intake of alcoholic beverages (7) in arteriosclerosis (3 10) in patients with von Gierke s disease (4) etc If blood lactate is elevated the decreased excretion of urate leads to hyperuricaemia

A similar effect can be achieved with ketone bodies such as beta hydroxybutyrate or acetoacetate It has also been shown that a high fat diet and starvation lead to diminished uric acid elimination resulting in hyperuricaemia (12)

Against this background it was interesting to study the influence of respiratory acidosis on serum uric acid levels and urate excretion in a small number of patients treated for respiratory insufficiency in the intensive therapy unit of the Department of Medicine University of Oulu

MATERIAL AND METHODS

The material consisted of seven consecutive patients admitted to the intensive therapy unit for respiratory acidosis Their clinical condition had deteriorated progressively over several days to such an extent that intensive therapy was necessary In three patients with chronic bronchitis and pulmonary emphysema the cause of severe deterioration was pulmonary infection One patient with generalized obstructive airway disease developed acute spontaneous pneumothorax which was the final cause of deterioration In three patients with bronchial asthma intense and prolonged obstruction led to severe alveolar hypoventilation and to clinical symptoms of CO retention

The series according to age sex and diagnosis is presented in Table I

The patients were treated according to currently accepted principles They received antibiotics bronchodilating agents expectorants corticosteroids and digitalis preparations if necessary Initially all patients received fluids parenterally and thereafter orally since adequate hydration

Table I *Distribution of seven patients admitted to the intensive therapy unit for respiratory acidosis by sex age and diagnosis*

Pat	Age	Sex	Diagnosis
K M	62	♀	Chronic bronchitis with emphysema
M P	18	o	Bronchial asthma
M J	67	o	Chronic bronchitis with emphysema
H H	63	♀	Bronchial asthma
G A	54	♀	Bronchial asthma
M V	51	♂	Spontaneous pneumothorax
P J	67	♂	Chronic bronchitis with emphysema

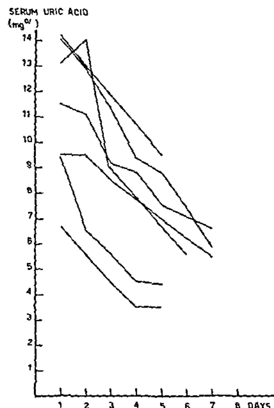


Fig 1 Serum uric acid (mg %) under treatment of respiratory acidosis

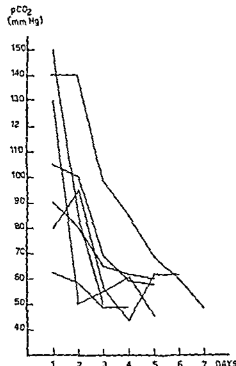


Fig 2 pCO (mm Hg) measured daily under treatment of respiratory acidosis

helps to liquefy the secretions and expedites their removal A locally humid atmosphere was also used to facilitate the loosening and expectoration of secretions In three cases the secretions were removed by aspiration through tracheostomy Use of intermittent positive pressure breathing also promoted bronchial drainage and further assisted the removal of secretions In all the cases intermittent positive pressure breathing (the Bennet Model PR 1 A or PR A) with 40% oxygen was used to blow off carbon dioxide The Astrup values using the capillary method (4) were analysed daily (or several times a day) The serum uric acid was measured daily in each patient as was the amount of uric acid excreted The measurements of uric acid were performed using the colorimetric Auto-Analyzer method (6) Facilities for determination of lactic acid were not available in this study The period of treatment and observation of patients in the intensive therapy unit varied from three to seven days

RESULTS

It can be seen from Figs 1 and 2 that improved alveolar ventilation leads to a progressive decrease in the high pCO values during treatment and high serum uric acid levels show a similar decline

Table II shows daily mean values of serum uric acid pCO blood pH and urinary uric acid of the patients

During the first day while the excretion of uric acid is still fairly low and serum uric acid high the blowing off of carbon dioxide and better oxygenation achieved by assisted ventilation and other treatment caused a considerable increase of urinary uric acid excretion and a progressive decrease in serum uric acid

Table II *The mean values of serum uric acid urinary uric acid blood pH and blood pCO_2 in seven patients with respiratory acidosis during intensive therapy*

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Serum uric acid (mg)	11 2	10 4	8 3	7 3	6 8	6 [illegible]	5 9
Urinary uric acid (mg/24 h)	351	573	660	580	415		
pH	7 223	7 259	7 342	7 422	7 378		
pCO_2 (mm Hg)	106	101	64	60	57		

DISCUSSION

Evidently respiratory acidosis leads to consider able hyperuricaemia though this finding has not been reported earlier in the literature All the present patients had hyperuricaemia of consider able magnitude prior to the initiation of intensive therapy for respiratory acidosis It appears that urinary elimination of uric acid is impaired under respiratory acidosis

Although in this series large quantities of uric a id were excreted even on the first day this was only an early result of effective therapy The pres ent results show that the amounts excreted almost doubled as respiratory acidosis decreased Al though hyperuricaemia in these patients cannot be attributed to diminished excretion alone excre tion increased remarkably as carbon dioxide and *simultaneously* the serum uric acid *fell to normal* levels

The results and findings reported earlier in the literature support the view that acidosis itself regardless of its origin leads to hyperuricaemia It has been shown for example that infusion of fru tose produ es an elevation of serum lactic acid and serum uric acid It also increases the urinary urate excretion (9) Similar increased uric acid formation has been shown to exist in one patient suffering from von Gierke s disease (1) In von Gierke s disease hyperuricaemia coexists with an elevated serum lactic acid and a decreased renal urate clearance (5)

The intake of alcoholic beverages results in lactic acidaemia and hyperuricaemia The small decrease of urinary urate excretion is insufficient to explain elevated serum values (8) Sorensen (15) has shown that uric acid can also be excreted into the gut In patients with renal insufficiency up to 70 of the uric acid is eliminated in this way (15) The influence of respiratory acidosis on this intestinal route of elimination has not been studied nor is it mentioned in the literature All the present patients had normal renal function and daily urinary volumes An essential cause of hyper uricaemia induced by respiratory acidosis is de creased urinary uric acid excretion This theory is also supported by the decreased renal urate clearances recorded in connection with infusions of lactic acid or ketone bodies However the pos sibility of increased uric acid formation as an es sential factor in these conditions has not been absolutely ruled out (9)

Uric acid ultimately consists of xanthine and hypoxanthine influenced by xanthine oxydase en zyme Seegmiller et al (13) described a syndrome of hyperuricaemia accompanying a congenital defect of the guanine hypoxanthine phosphoribo syltransferase enzyme Uric acid formation is greatly increased in these patients although the exact mechanism of the increase is not fully under stood Thus these enzymes could be influenced by acidosis so as to increase the produ tion of uric acid

In patients with myocardial infarction and with cerebrovascular damage (3 14) the hyp ruricaemia may be largely due to coexistence of metabolic acidosis known to occur under these conditions However it has been shown that in patients with myocardial infarction the serum uric acid remains *at an elevated level for a considerable period of* time even after the acidosis has disappeared (14)

In secondary hyperuricaemia clinical manifesta tions may also occur in the form of acute gouty arthritis in connection with sudden changes of serum uric acid levels (8) both in haemodialysis and starvation A patient unconnected with the present series who had severe respiratory acidosis and high serum uric acid contracted an acute at tack of gouty arthritis on the sixth day of treat ment His serum uric acid had fallen from 11 6 to 5 7 mg ₀ The typical attack involved the metatarsophalangeal joint of the big toe Even *in secondary hyperuricaemia produced by respira* tory acidosis it remains obscure why an acute at tack of arthritis should manifest itself occasionally when the uric acid levels are remarkably low and why in other cases no clinical symptoms occur despite greatly elevated serum levels

REFERENCES

1 Alepa F P Howell R R Klinenberg, J R. & Seeg miller J E Relationship between glycogen storage disease and tophaceous gout Amer J Med 4? 58 1967

Astrup P A new approach to acid base metabolism *Clin Chem. 7 1 1961*

3 Hansen, O E Hyperuricemia in cerebral infarction Acta p ychiat scand Suppl 13 1965

4 Howell R. R The interrelationship of glycogen storage disease and gout Arthr and Rheum 8 780 1965

5 Hoyingen Huene C B J von Gout and glycogen storage disease in preadolescent brothers Arch. intern med 118 471 1966

6 Kuhlback B & Widholm O Serum uric acid in toxaemia of pregnancy with special reference to the prognosis of the foetus Acta obstet gynec scand 43 330 1965
7 Lieber C S Hyperuricemia induced by alcohol Arthr and Rheum 8 786 1965
8 Maclachlan M J & Rodnan G P Effects of food fast and alcohol on serum uric acid and acute attacks of gout Amer J Med 42 38 1967
9 Perheentupa J & Raivio K Fructose induced hyper uricemia Lancet 2 528 1967
10 Schrade W Boehle E & Biegler R Humoral changes in arteriosclerosis Lancet 2 1409 1960
11 Scott J T Factors inhibiting the excretion of uric acid Proc roy Soc Med 59 310 1966
12 Scott J T McCallum F M & Holloway V P Starvation ketosis and uric acid excretion Clin Sci 27 209 1964
13 Seegmiller J E Rosenbloom F M & Kelley W N Enzyme defect associated with a sex linked human neurological disorder and excessive purine synthesi Science 155 1682, 1967
14 Spring M Cavusoglu M Chu Y C & Artymowska C Hyperuricemia and hyperglycemia in acute myo cardial infarction Circulation 22 817 1960
15 Soren en L B Role of the intestinal tract in the elimination of uric acid Arthr and Rheum 8 694 1965
16 Uric acid method In Autoanalyzer manual Tech nicon Instruments Corp Chauncey New York 1960
17 Weiner I M & Mudge G H Renal tubular mechan ism for excretion of organic acids and bases Amer J Med 36 743 1964
18 Yu T F Sirota J H Berger L Halpern M & Gutman A B Effect of sodium lactate infusion on urate clearance in man Proc Soc exp Biol (N Y) 96 809 1957
19 Zachau-Christiansen B Rise in serum uric acid dur ing muscular exercise Scand J clin Lab Invest 11 57 1959

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APPARENT RESISTANCE TO ORAL ANTICOAGULANT THERAPY AND INFLUENCE OF HYPNOTICS ON SOME COAGULATION FACTORS

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Abstract The influence on the one-stage prothrombin time (Quick index) prothrombin + proconvertin activity platelet count and fibrinogen level of a barbiturate Diminal duplex® (Astra) (10 patients) and of a non barbituric hypnotic drug Placidyl® (Abbot) (6 patients) was studied during treatment with bishydrocoumarin AP® (Ferrosan) An increase in the Quick index was noted in both groups whereas the prothrombin + proconvertin activity fibrinogen level and platelet count were unaffected The dose response of bishydrocoumarin on Quick index was studied in 20 patients Two of these did not respond to large doses of bishydrocoumarin Other anticoagulants such as Waran® Sintroma® and Tromexan® were also ineffective when given orally Intravenous warfarin (Waran®) effectively lowered the Quick index in one of the patients but not the other One patient resistant to bishydrocoumarin successfully treated with Marcoumar orally is also described Apparent resistance to oral anticoagulants during treatment with hypnotic drugs and different causes of anticoagulant resistance per se are discussed

During treatment with coumarin derivatives the patients are often given other drugs A decreased prothrombin response after administration of barbital compounds has been reported (2 8 9 21) The purpose of the present investigation was to study the effect of certain drugs on blood coagulation estimated by platelet count prothrombin + proconvertin activity one-stage prothrombin time (Quick index) and fibrinogen level

MATERIAL AND METHODS

Patients being given anticoagulant therapy (bishydrocoumarin AP® Ferrosan Sweden) were studied they were free from known liver kidney or gastrointestinal disease and were fed on a regular hospital diet. Only those patients were included who after at least one week s dicoumarol treatment had a stabilized therapeutic" Quick index or prothrombin + proconvertin (P + P) activity No hypnotics were given for a week before the investigation Bishydrocoumarin and the relevant drug were administered at 8 p m Blood specimens were drawn every day at 8 a m A fixed dose of bishydrocoumarin was given during the 23-day experimental period The influence was studied of two hypnotics—i e Diminal duplex® (vinbarbital sodium 100 mg and aprobarbital 50 mg Astra Sweden) and Placidyl® (3-(2-chlorovinyl)penten-(1) tol Abbot)—on the platelet count prothrombin + proconvertin activity one stage prothrombin time (Quick index) and fibrinogen level Other apparent resistance to treatment with coumarin derivatives was also noted

The one-stage prothrombin time (Quick index) was determined as described by Lehman (12) The platelet count was determined according to Kristensson (11) and prothrombin + proconvertin activity according to Owren and Aas (20) Fibrinogen was determined by the method of Bergstrom et al (3)

RESULTS

Ten patients with deep venous thrombosis and a Quick index of about 30% were given two tablets of Diminal duplex® daily The Quick index and prothrombin + proconvertin activity were recorded After 18 days administration of the hypnotic the Quick index rose from an average 36% to 53% (Fig 1) Although the prothrombin + proconvertin activity rose in six patients no significant changes occurred No consistent changes were noted in platelet count and fibrinogen level When hypnotic therapy was discontinued the Quick index slowly returned to the normal level within about a week Thus barbiturate administration was associated with an increase of the Quick index

After six patients with myocardial infarction had been treated for two weeks with bishydrocoumarin they were given concurrently a daily dose (1 g) of Placidyl® as a hypnotic During the subsequent 18 days the Quick index rose from an average 38% to 55% (Fig 2) No significant change took place in the prothrombin + proconvertin activity fibrinogen level or platelet count

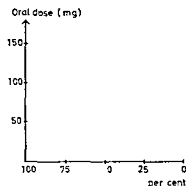


Fig 1 Oral do e of bishydrocoumarin (mg) and Quick index ( ) before (●) and after (O) concurrent ad ministration of a barbiturate (Diminal duplex® Astra)

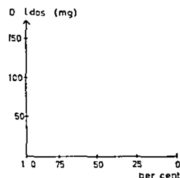


Fig 2 Oral dose of bishydrocoumarin (mg) and Quick index ( ) after two weeks therapy (●) and after 18 days concurrent administration of 1 g of Placidyl® (Abbot) daily (O)

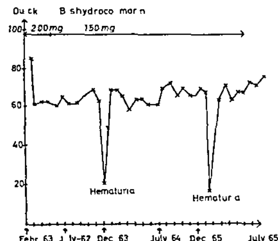


Fig 3 Quick index ( ) in a 6_ year-old man (O B) treated after a myocardial infarction with bishydrocoumarin and a regular evening dose of Placidyl® (1 g)

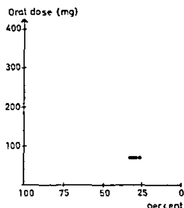


Fig 4 Quick index ( ) in 20 patients treated with bis hydrocoumarin

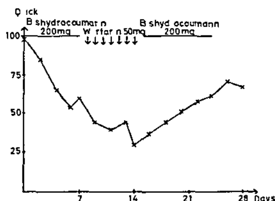


Fig 5 Resistance to oral and intravenous anticoagulant therapy

Resistance to coumarin therapy was noted in one patient He was a 62 year old man (O B) who following a myocardial infarction was treated with bishydrocoumarin (AP®) and reg ularly took Placidyl® (1 g) every evening as a hypnotic The dose of anticoagulant and his Quick index are shown in Fig 3 Two episodes of haematuria occurred his Quick index—which was normally high despite large doses of AP—fell con currently to 21° and 14% When the bleeding occurred he had been without Placidyl for 4 and 6 days respectively

Fig 4 shows the relation between the Quick index and the oral dose of bishydrocoumarin in 20 patients They were treated with the anti coagulant for one week and the same dose was given for seven days before starting the investiga tion It is remarkable that two of these patients did not respond to large doses of bishydro- coumarin One was a 68 year old woman (A E)

with a history of at least two pulmonary thrombo embolisms The other was a 61 year-old man (G O) with a deep thrombosis of the calf The lowest Quick index recorded during the whole treatment period was 52° in the woman and 58 in the man In these two patients other coumarin derivatives such as warfarin (Waran®) (Nyegaard) Sintroma® and Tromexan® (Geigy) were also ineffective when given orally Intra venous injection of 50 mg of Waran reduced the Quick index in the man (Fig 5) but had no effect in the woman (Fig 6)

Fig 7 shows resistance to bishydro oumarin ap pearing in a 58 year old woman (S A) with myo ardial infarction A change to Marcoumar® (Roche) successfully lowered her Quick index

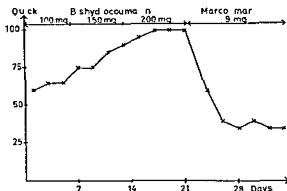


Fig 7 Quick index ( ) in a 58 year-old woman (S A) with myo ardial infarction during treatment with bis hydrocoumarin and Marcoumar Resistance to the former appeared during treatment

DISCUSSION

Avellaneda (2) and Grilli (7) observed a decrease in the prothrombin time after addition of various barbiturates in patients treated with ethyl biscoum acetate Reverchon and Sapir (21) found that the dose of this anticoagulant had to be increased when barbiturates—of which they often prescribed extremely large doses—were given concurrently The present study shows that administration of a barbiturate and of Pla idyl® in combination with another dicoumarol compound—bishydrocoumarin (AP®)—seemed to increase the Quick index but did not change the P+P activity The same ob servation on the P+P level after barbiturate in take was made by Samuelsson and Lilienberg (22)

The coumarin anticoagulants influence the clot ting mechanisms in the same way by interfering in the synthesis of clotting factors II VII IX and X Since barbiturates influence the Quick index

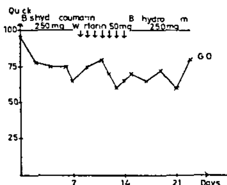


Fig 6 Resistance to oral anticoagulant therapy

but not the prothrombin+proconvertin activity fibrinogen level or platelets it is possible that they could influence factor X

The mechanism of barbital action on the pro thrombin complex is unknown Enzyme stimula tion was suggested by Weiner (25) and Cucinell et al (5) An increased activity of liver enzymes which metabolize dicoumarol has in fact been noted in rats after phenobarbital treatment Day ton et al (6) showed that pretreatment with hep tabarbital lowered the plasma drug level of di coumarol and diminished the decrease in the pro thrombin response when dicoumarol was given orally but not when administered intravenously Aggeler and O Reilly (1) demonstrated that barbital decreased the absorption of dicoumarol from the gut to the blood and that greater amounts of the drug could be detected in the stools They also noted that the barbital effect on the dicoumarol persisted for some weeks after withdrawal of the hypnotic

When such drugs are administered larger doses of the oral anticoagulant seem to be necessary

Apparent resistance to the hypoprothrombin aemic effect of therapy with coumarin derivatives has been reported in man and in animals (17 24) both as an acquired phenomenon and as an inherited trait (19) Hyperlipaemia (10) gastro intestinal disorders (23) pulmonary infarction (18) lactation (13) and con omitant administration of other drugs (13) may also produce such resistance to coumarin therapy

The present study supports the concept of dif ferent mechani ms of coumarin resistance

The findings of resistance to oral but not to intravenous therapy (Fig 5) are in line with the view that barbiturates decrease the absorption of coumarin derivatives presumably due to influence on an active absorption mechanism

Spontaneous resistance to dicoumarol and warfarin occurred in this material as described earlier One possible explanation is an extremely rapid metabolism earlier demonstrated by O Really et al (19)

In investigated cases the Quick index measuring the coagulation factors II VII IX and X was increased whereas the prothrombin + proconvertin actively measuring only the factors II and VII was unchanged The inference is that in contrast to earlier explanations the barbiturates (and placidyl) do not influence the metabolism of dicoumarol derivates but their effect on the factors IX and X of the coagulation system

REFERENCES

1 Aggeler P M & O'Reilly R A Pathogenesis and treatment of thromboembolic diseases p 277 F K Schattaner Verlag Stuttgart 1966
2 Avellaneda M Medicina (B Aires) 15 109 1955
3 Bergstrom K Blomback B & Kleen G Acta med scand 168 291 1960
4 Carter S A New Engl J Med 273 423 1965
5 Cucinell S A Conney A H Sansur M & Burns J J Clin Pharmacol Ther 6 420 1965
6 Dayton P G Tarcan Y Chenkin T & Weiner M J clin Invest 40 1797 1961
7 Grilli H Pren méd argent 46 2867 1959
8 Johansson S A In Udden P Svenska Lak Tidn 60 21 1963
9 — In Samuelsson S M & Lilienberg K Scand J clin Lab Invest 17 77 1964
10 Jones R J & Cohen L Circulation 24 134 1961
11 Kristensson K Acta med scand 69 127 1928
12 Lehman J Mschr Kinderheilk 84 44 1941
13 Levine W L Anticoagulants In The pharmacological basis of therapeutics (ed L S Goodman & A Gilman) p 1453 McMillan Co New York 1965
14 Lewis J L Spivac M & Spaet T H Amer J Med 42 620 1967
15 Loedinger E A Praxis 73 917 1961
16 Lowenthal, I & Fischer L M Experientia (Basel) 13 253 1957
17 Lund M Nature 203 778 1964
18 Olwin J H In Thrombosis and embolism (ed T Koller & W R Merz) p 713 Benno Schwalbe & Co Basel 1955
19 O Reilly R A Aggeler P M Hoag M S Leong L S & Kropatkin M L New Engl J Med 271 809 1964
20 Owren P A & Aas K Scand J clin Lab Invest 3 201 1951
21 Recerchon F & Sapir M Presse méd 69 1570 *1961*
22 Samuelsson S M & Lilienberg K Scand J clin Lab Invest 17 72 1964
23 Soulier J P Path et Biol 8 985 1960
24 Wanntorp H Acta pharmacol (Kbh) Suppl 2 1 1959
25 Weiner M In Seminars in hematology (ed P A Meischer) vol 1 p 345 Grune and Stratton New York 1964

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COBALT INDUCED HYPOTHYROIDISM AND POLYCYTHEMIA IN LIPOID NEPHROSIS

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Abstract An 11 year-old boy who had been suffering from genuine lipoid nephrosis from the age of 6 / years received thiosemicarbazone for three weeks During this therapy severe anemia developed. The anemia was treated with cobaltous chloride During the fourth month of therapy the child developed very severe hypothyroidism There was considerable enlargement of the thyroid gland along with moderate polycythemia These events are thought to be due to the cobalt therapy

In 1929 Waltner and Waltner (48) observed that administration of cobalt salts to rats produced polycythemia This erythropoietic effect of cobalt salts has been repeatedly confirmed Consequently cobalt has been used for the treatment of various refractory types of anemia including the nephrogenic form (3 13 22) Massino and Pantarotto (29) reported a marked increase in erythropoietin concentration in the blood of azotemic patients during cobalt treatment This increase in erythropoietin was accompanied by an improvement in the anemia

Genuine lipoid nephrosis resistant to treatment with corticosteroids is still a therapeutic problem Heilmeyer (17) originally suggested that thiosemicarbazone (TSC) could be used in the treatment of the nephrotic syndrome and since then it has been used in this disease by many authors

In this paper a case of genuine lipoid nephrosis in a male child will be presented During TSC therapy severe anemia developed and this was treated with iron and cobaltous chloride In the course of this treatment polycythemia goitre and hypothyroidism developed

CASE REPORT

The patient was a 1 year-old boy whose brother had suffered from an edematous renal disease from which, however he had recovered No thyroid diseases had occurred in the family The patient was in good health until the age of 6 years (in 1961) when he had an acute infection followed by typical signs of lipoid nephrosis He was initially treated with corticotrophin (ACTH) and corticosteroids in a local hospital but as there was no improvement in his condition he was transferred to the Children s Hospital University of Helsinki At that time he had abundant proteinuria (ad 1 4) electrophoresis of the serum proteins revealed a decrease in albumin and gamma globulin and an increase in alpha ? globulin The serum cholesterol was elevated the highest value being 1030 mg 100 ml The ESR was 135 mm h and the BP 150 100 mm Hg He was again initially treated with ACTH and later with prednisone intermittently Attempts were made to increase the urine excretion with different diuretics but only spironolactone was effective During this treatment there was clinical improvement and a concomitant improvement in the laboratory tests After discharge from hospital treatment was continued with ACTH prednisone and diuretics For the next two years the patient was followed up at the outpatient department and was temporarily hospitalized a few times The nephrotic signs however persisted in varying degree

In April 1963 a Cushingoid syndrome developed and prednisone treatment was stopped

In October 1963 he was again hospitalized since the edema had increased After a short course of prednisone the treatment was continued with chlorthalidone alone His condition deteriorated but treatment with corticosteroids was not started because the patient had been exposed to chickenpox Instead TSC treatment was introduced (75 mg three times a day) After two weeks of treatment the patient became tired complained of nausea and began to vomit His Hb had decreased from 12.8 to 7 5 g 100 ml X ray pictures of the long bones showed changes compatible with leukemic infiltrations The white blood picture however was normal The bone marrow smear indicated the possibility of hemolytic (uremic) anemia and since the blood urea was elevated (63 mg 100 ml) this explanation seemed to be most likely The patient was given blood transfusions and treatment with cobaltous chloride and iron was introduced The daily dose of CoCl was 10 mg and of iron 44 mg At the same time the patient got mumps, which ran an uneventful course He was discharged and treated at home with anabolic steroids and cobalt iron



Fig 1 The patient after four months of cobalt therapy Note the enlargement of the thyroid gland

In March 1964 after three months of this treatment he b came increasingly tired and complained of pain in the chest dyspnea occurring even after moderate exercise and a feeling of compression in the neck He became slow and somnolent and his skin was dry and coarse On physical examination the reflexes were sluggish and the thyroid previously normal in size was now estimated by palpation to be about 100 g (Fig 1) There was no tenderness The blood picture showed marked polycythemia (Hb 18 6 g/100 ml RBC 5 78 mill/mm³) Bone marrow biopsy revealed no abnormalities The PBI was rep atedly low (1 3 to 1 8 gamma/100 ml) and the BEI was 0 4 gamma/100 ml The serum cholesterol was 15 0 mg 100 ml No thyroid antibodies (hemagglutination and complement fixation tests) were present No LE cells were found and the antinuclear factor was negative Anti strepto and antistaphylolysin titers were normal The ECG showed low voltage amplitudes Because of the very low cardiac output the pulse rate was however relatively high After treatment with thyroxine there was rapid change in the voltage and pulse rate (Fig. 2) Intravenous pyelography revealed slow excretion of dye but no evidence of renal tumour Histological examination of a specimen of thyroid tissue taken surgically showed an extremely activated histological picture The epithelium was very high and most follicles were devoid of colloid There were no signs of malignancy or of giant-cells or lymphoid thyroiditis (Fig 3) Althou h cobalt treatment was discontinued immediately the patient had severe spells of tiredness and fatigue durin which the BP decreased to 95/90 For this reason radioactive iodine tests could not be carried out before starting treatment with thyroxine

Treatment with thyroxine 55 μg a day was begun and within nine days the patient s condition had improved considerably whereupon the thyroxine dose was increased to 110 μg a day After three weeks treatment the serum cholesterol decreased to 640 mg/100 ml and the thyroid returned to normal size Throughout this period the nephrotic syndrome was treated with diuretics only

The patient was discharged in May 1964 in fairly good condition On repeated follow up examinations since then he has been doing well on thyroxine and diuretics The PBI has varied from 3 1 to 6 8 gamma/100 ml the serum cholesterol from 398 to 844 mg/100 ml the blood urea from 19 to 37 mg/100 ml and the proteinuria from 0 17 to 0 80 The size of the thyroid has remained normal

DISCUSSION

It was primarily assumed that the patient presented in this paper had anemia of renal origin Leakage of transferrin into the urine has been suggested (6) to cause iron deficiency in nephrogenic anemia but the possibility of faulty synthesis of this carrier protein cannot be dismissed *Gitlin* et al (14) found synthesis to be normal but

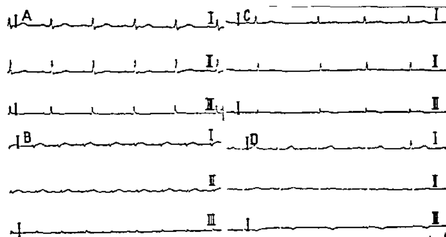


Fig 2 ECG (A) Before the hypothyroid state (B) During the hypothyroid period (C) Six days after introduction of thyroid therapy (D) Twelve days after introduction of thyroid therapy I = 1 mV Note the changes in the amplitude and pulse rate

showed that nephrogenic anemia may be due to leakage and to increased catabolism of transferrin Hen e it is possible that in the present case iron stores were decreased

Development of anemia has been observed during treatment with thiosemicarbazone (TSC) (41) Bone marrow changes have been non specific without any qualitative alterations (41) Pribilla and Koester (34) reported six cases of hemolytic crises during TSC therapy

It seems probable that the anemia in this case was produced both by the action of TSC and by the increased catabolism and leakage of transferrin

That cobalt stimulates erythropoiesis is a well established fact but the exact mechanism is still obscure It has b en claimed that the hypoxia in bone marrow caused by cobalt releases red cells from the marrow (2) but other investigators (25) have been unable to confirm these findings Cobalt is known to interfere with tissue respiration (9 46) and it is suggested that the hypoxia thus created stimulates erythropoietin production (4 10) Whether this is related to cytochrome oxidase activity (42) or to a renal erythropoietin inhibiting factor (11) is not known It might be that there are other sites of erythropoietin production apart from the kidney (45)

Orten (30) suggested that the vasodilatation caused by cobalt results in local anoxia and it has later been shown (20) that the erythropoietin stimulating effect of cobalt may be related not only to histotoxic anoxia but also to stagnant hypoxia following a severe decrease in cardiac output

During treatment with cobalt polycythemia developed in our patient It seems likely that this was a result of the treatment with cobalt. The possibility that the anabolic steroid given during the same period as the cobalt contributed to the development of this condition is not ruled out. Polycythemia probably due to increased erythropoietin production has been described in various renal diseases (12) but the presence of anemia in our patient before the development of polycythemia argu s against this possibility

Cobalt has been reported to induce a variety of adverse reactions of which anorexia nausea vomiting and diarrhea are the most common (3 13) Others include erythema and hot sensations (3) skin rashes (51) and substernal aches (3)

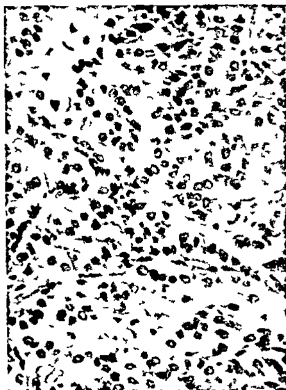


Fig 3 Microscopic appearance of the thyroid gland A surgical biopsy HE, × 240 Note the high epithelium and the practically total lack of colloid

Further serious complications are tinnitus and neurogenic deafness (13)

The effect of cobalt salts on the thyroid was first reported by Gross et al (16) who obs rved the development of goitre and hypothyroidism in children given cobaltous chloride for the treatment of anemia but the idea that cobalt might be a thyrostatic agent was initially challenged by other authors (19) Since then however cases have been reported in which goitre and hypothyroidism have developed during treatment with cobalt (7 49) Roche and Layrisse (39) observed a significant decrease in the thyroidal uptake of ^{131}I in eu thyroid subjects after two weeks treatment with cobaltous chloride the uptake was in fact almost totally blocked Pimentel Malaussena et al (33) observed a therapeutic effect in hyperthyroid patients as well as decreased thyroidal ^{131}I uptake after treatment with cobalt These effects have been confirmed by other authors (31) Thiocyanate has been reported to cause an outpouring of radioactive iodine during cobalt administration indicat

ing the presence of a binding defect in the thyroid (31) The goitrogenic effect of cobalt in man thus seems to have been amply demonstrated

The findings in experimental animals however have been more controversial In studies with guinea pigs Anttila et al (1) observed the production of goitre histological activation of the thyroid and a decrease in PBI after cobalt administration whereas similar studies in rats mice rabbits and young chicks have produced unequivocal results In 1 day old chicks however Kriss et al (24) were able to produce a decrease in the thyroidal uptake of ^{131}I and an increase in the uptake of ^{32}P but no significant colloid depletion TSH and propylthiouracil (PTU) induced very marked colloid depletion TSH is known to increase the thyroidal uptake of ^{32}P in from 1 to 3 day old chicks (26) and to produce changes in the colloid epithelium ratio The same was observed in the study of Kriss et al (24) Administration of PTU to rats induces histological activation as well as an increase in the uptake of ^{32}P by the thyroid (47) Kriss et al (24) drew the conclusion that because of the discrepancy between the effect of cobalt on the uptake of ^{32}P and colloid depletion as compared to that of PTU the mode of action of cobalt on thyroidal iodine metabolism was not similar to that of PTU It must be borne in mind however that the uptake of ^{32}P in the thyroid reflects general cellular activity and epithelial growth (27) Hence it stands to reason that whatever the precise mode of action of cobalt might be the ion must induce some kind of blocking of intrathyroidal iodine metabolism which in turn and in the same manner as other goitrogenic substances leads to goitre through the mediation of TSH However this has not been further explored by newer methods of investigation Blocking of the protein binding of iodine seems to occur anyway

As regards the mode of action it has been observed by Levy et al (28) that the activity of some SH dependent enzymes such as succinoxidase and cholinoxidase as well as that of the non SH dependent cytochrome oxidase and catalase is inhibited by cobalt in an aerobic milieu This may have some bearing at least on the hematological effects of cobalt (43) but it is also known that other inhibitors of cytochrome oxidase such as cyanide azide sulphide and carbonmonoxide inhibit the formation of diiodothyrosine and thyroxine (40) Some studies of Kirkwood and Fawcett have been quoted by Jaimet and Thode (19) and Kriss et al (24) according to which cobalt in a 10^{-3} *M* concentration exerts an inhibiting effect on thyrosine iodination in a cell free system

It has been suggested that the effect of cobalt on the thyroid might be some kind of allergic reaction (7) Furthermore the idea has been advanced (49) that some kind of hereditary primary defect in thyroid iodine metabolism must exist before cobalt can exert its goitrogenic effect This is thought to hold good as far as iodine induced goitrous hypothyroidism is concerned and of course if such a defect is present any kind of goitrogen may produce evident thyroid manifestations no matter how small the dosage This however is still far from settled

Different results obtained by various workers may merely reflect difference in dosage in intestinal absorption in iodine supply etc In fact the intestinal absorption of cobalt especially if administered after a meal seems to vary greatly (32) Cobalt is rapidly absorbed and disappears rapidly from the blood which makes the blood concentrations vary widely Most cases of cobalt induced goitrous hypothyroidism have been reported in children The different rates of occurrence in children and adults may be due to different dosage schedules In adults one is used to a definite steady daily dosage and only two cases of goitrous hypothyroidism in adults have been reported (both with chronic renal disease) In children cobalt is administered according to body weight which might result in blood levels relatively higher than in adults It is conceivable that faulty renal excretion may be of importance In the case reported here the glomerular filtration rate was substantially decreased because of the primary disease and the TSC therapy (41)

The role of TSC in the development of goitre and hypothyroidism in this case is unknown The formula of TSC bears some resemblance to that of the thiocarbamides Corti and Romualdi (8) found histological changes in the thyroid of rats after TSC treatment resembling those found after thiocarbamide treatment but it has been reported only once (44) that TSC may have caused goitre In the present case the goitre appeared fairly rapidly after introduction of cobalt therapy but the additional affect of the preceding TSC treatment cannot be excluded

The hypercholesterolemia and the decreased basal metabolic rates originally suggested that the thyroid function in the nephrotic syndrome might be impaired This in turn might have some bearing on the nephrosis itself The PBI has also occasionally been found to be low Thyroid therapy has been tried but without improvement of the clinical condition or the PBI (21) Holsti (18) described a hypothyroid patient in whom nephrosis developed and in this case thyroid treatment clearly improved the nephrotic condition In the present case there was initially a certain degree of improvement after thyroxine treatment was introduced but it is quite possible that remission was only temporary The low PBI found in nephrosis is thought to be due to a low level of binding sites in the thyroxine binding globulin (TBG) in the serum (38) resulting from leakage of this carrier protein into the urine (21 35) There is usually no histological alteration in the thyroid gland in patients suffering from the nephrotic syndrome (36) although in one case change have been observed (50) There might however be a decrease of iodine stores in the thyroid since Recant and Riggs (36) found a lowered response to TSH in nephrotic patients after methimazole treatment. In the present case the PBI was low but determination of the TBG could not be carried out

In this case serum cholesterol elevation is thought to be the result both of the nephrotic syndrome and of hypothyroidism Cobalt on the other hand is known to have a lipogenic effect (5) This effect has been postulated to be due to a lipid mobilizing hormone (53) the concentration of which has been reported to be increased in the blood of patients with the nephrotic syndrome (52)

The patient described here had mumps immediately before the hypothyroidism occurred but thyroid biopsy and the absence of thyroid antibodies in the blood seem to exclude the possibility that subacute thyroiditis was the cause of the events

We submit therefore that the hypothyroidism and goitre in this case were due to the administration of cobalt for treatment of the anemia produced by TSC

REFERENCES

1 Antila, V., Telkka A. & Kuusisto A N Acta endocr (Kbh.) 20 351 1955
2 Barron A G & Barron, E S G Proc Soc exp Biol (N Y) 35 407 1936–37
3 Berk, L Burchenal J H & Castle W B New Engl J Med 240 754 1949
4 Brown, T E & Meincke H A Proc Soc exp Biol (N Y) 99 435 1958
5 Caplan, R. M & Block W D J invest Derm. 40 199 1963
6 Cartwright G E Gubler C J & Wintrobe M M J clin Invest 33 685 1954
7 Chamberlain, J L. J Pediat 59 81 1961
8 Corti L & Romualdi G Folia endocr (Roma) 3 139 1950
9 Dingle J T & Heath J C Biochim biophys Acta (Amst.) 65 34 196
10 Fisher J W & Birdwell B J Acta haemat (Basel) 26 224 1961
11 Fisher J W Porteous, D D Hirashima K. & Tso S C Proc Soc exp Biol (N Y) 12? 1089 1966
12 Forsell J Acta med scand 161 169 1958
13 Gardner F H J Lab clin Med 41 56 1953
14 Gitlin D Janeway C & Farr L J clin Invest 35 44 1956
15 Grant W C & Root W S Physiol Rev 3? 449 195?
16 Gross R T., Kriss J P & Spaet, T H Amer J Dis Child 88 88 1954
17 Heilmeyer L Dtsch med Wschr 76 955 1951
18 Holsti O Acta med scand Suppl 196 ?85 1947
19 Jaimet C H & Thode H G J Amer med Ass 158 1353 1955
20 Jalavisto E., Makkonen, E., Makkonen H & Pallasvuo M R Ann Acad Sci fenn A 115 1 1965
21 Kalant N., McIntyre W C & Wilansky D L Endocrinology 64 333 1959
22 Kasanen A A Kulonen E I & Iisalo E I Acta med scand 171 379 196?
23 Kohler H Dtsch Gesundh Wes 11 169 1956
24 Kriss J P Greenspan F S Carnes W H & Lew W Endocrinology 59 555 1956
25 Laforet, M T & Thomas E D J biol Chem 218 595 1935
26 Lamberg, B A Acta med scand Suppl 279 1953
27 — Acta endocr (Kbh) 18 405 1955
28 Levy H V Levison V & Schade A L Arch Biochem 27 34 1950
29 Massino L & Pantarotto M F Minerva pediat 15 1 1963
30 Orten J M Amer J Physiol 114 414 1936
31 Paley K R Sobel E S & Yalow R S J clin Endocr 8 850 1958
32 Paley K. R. & Sussman, E Metabolism 1? 975 1963
33 Pimenthel Malaussena E., Roche M & Layrisse M J Amer med Ass 167 1719 1958
34 Pribilla W & Koester E D Dtsch med Wschr 9/30 795 1949
35 Rasmussen, H J clin Invest 35 79? 1956

36 Recant, L. & Riggs D S J clin Invest 31 789 1952
37 Robbins J., Rall, J E & Petermann M J clin Invest 36 1333 1957
38 Robbins J & Rall J E Physiol Rev 40 415 1960
39 Roche M & Layrisse M J clin Endocr 16 831 1956
40 Schachner H., Franklin, A L. & Chaikoff I L J biol Chem 151 191 1943
41 Schaich W Stadler L & Keiderling, W Beitr Klin Tuberk. 104 465 1951
42 Schultze M O J biol Chem 138 219 1941
43 Smith C H Blood disease of infancy and childhood p 44 C V Mosby Co., Saint Louis 1966
44 Stadler L & Weissbecker L Ärztl Wschr 6 2? 1951
45 Stohlman J Jr New Engl. J Med 267 342 & 39? 1962
46 Strickland E H & Gougher C R. Nature (Lond) 198 790 1963
47 Tala P., Lamberg B A & Uotila U Acta endocr (Kbh) 19 255 1955
48 Waltner K. & Waltner K. Klin Wschr 8 313 1929
49 Washburn T C & Kaplan E Clin Pediat 3 89 1964
50 Wolbach B & Blackfan K. Amer J med Sci 180 453 1930
51 Wolf J & Levy I J Arch intern Med 93 387 1954
52 Zarafonetis C J D Seifter J Baeder D H & Kalas J J Lab clin Med 50 965 1957
53 Zarafonetis C J D Barlett R. H & Brody G L J Amer med Ass 191 235 1965

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RELATION OF TUBULAR MAXIMUM REABSORPTION OF GLUCOSE AND PARATHYROID FUNCTION IN GOATS

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Abstract A well-defined tubular maximum reabsorption of glucose is demonstrated in goats The reabsorptive capacity is not affected by spontaneous changes of the glomerular filtration rate Administration of EDTA or parathyroid extract led to increased tubular reabsorptive capacity for glucose while calcium administration or parathyroidectomy had the opposite effect These observations support the previous assumption of a positive relationship between the tubular reabsorptive capacity for glucose and the parathyroid function

Recent observations in humans indicate a positive correlation between tubular reabsorptive capacity for glucose and parathyroid function (1 2) Increased values of the ratio TmG/GFR (that is tubular maximum for glucose related to the glomerular filtration rate) were found in patients with hyperparathyroidism and decreased values in patients with hypoparathyroidism compared to the values obtained in patients with no signs of endocrine disorders

The purpose of this report is to demonstrate a well-defined tubular maximum reabsorption of glucose in goats and to relate changes of TmG to induced alterations of the parathyroid function

MATERIAL AND METHODS

Four young female goats weighing [illegible]4 to 43 kg, were used for the investigation In three goats (A B and C) a glucose titration study that is determination of tubular reabsorption of glucose under increasing filtered load of glucose was performed to decide whether or not the goat has a well-defined TmG TmG was determined twice at intervals of 4 to 8 days in three goats (B C and D) directly by rapid elevation of glucose concentrations in blood Goat C was rendered hypercalcemic for [illegible]4 hours by i v injections of calcium levulate 60 mg/kg, at intervals of four hours and a direct TmG determination was performed. One week later the goat was given parathyroid extract (100 U.S.P units per ml E Lilly & Co) i.m. [illegible]00 I U a day for two days [illegible]00 I U twice on the 3rd day and [illegible]00 I U early in the morning of the 4th day when another direct TmG determination was carried out

In goat D infusion of a solution in water of 0 6% NaCl and 0 83% ethylene diamin tetraacetic acid (EDTA) was administered i v for two hours at a rate of [illegible] ml/min, followed by a direct TmG determination One week later the goat was parathyroidectomized, and on the second postoperative day another direct TmG determination was carried out.

By glucose titration the tubular reabsorption of glucose is determined under increasing filtered loads of glucose The fasting goat was anesthetized with nembutal (30 mg/kg i v) intubated, and put on a respirator with atmospheric

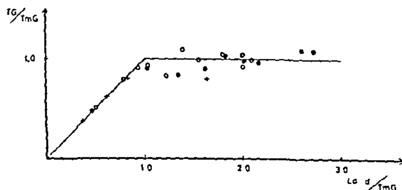


Fig 1 Glucose titration curves in three normal goats demonstrating a maximal tubular reabsorptive capacity for glucose *TG* tubular reabsorption of glucose (mg/min) *TmG* maximum reabsorption of glucose (mg/min) *Load* filtered load of glucose (mg/min)

Table I *Glucose titrations and TmG determinations in three normal goats*

	BG (mg/100 ml)	GFR (ml/min)		TG (mg/min)
Goat A (67–143)				
Glucose titration	68	120 6		82 0
	121	124 4		148 9
	186	119 8		211 2
	252	112 2		211 9
	335	113 8		209 6
	435	118 8	115 0	250 8
	476	112 5		232 2
	TmG = 230 9 mg/min			
Goat B (67–169)				
Glucose titration	74	128 1		92 6
	140	125 3		172 2
	184	117 5		183 2
	242	116 4		175 3
	308	112 1		187 3
	348	110 1		217 1
	420	101 8		206 6
	468	95 4	99 1	200 0
	526	88 7		200 0
	588	94 3		243 3
	632	91 1		237 4
	TmG = 211 1 mg/min			
Direct TmG determination	554	99 4		228 2
	584	92 6	96 8	215 3
	606	95 8		225 7
	614	99 3		223 4
	TmG = 223 2 mg/min			
Direct TmG determination	610	90 0		200 0
	640	80 9	82 2	206 2
	662	84 8		235 0
	698	73 1		204 5
	TmG = 211 4 mg/min			
Goat C (67–138)				
Glucose titration	84	77 4		64 8
	113	112 5		124 1
	159	89 1		127 1
	198	95 9		154 6
	233	71 3		114 3
	282	77 0		134 6
	319	87 0		144 2
	364	76 2	73 2	128 2
	402	71 5		137 0
	457	54 4		146 0
	TmG – 138 0 mg/min			

BG = blood glucose concentration GFR = inulin clearance TmG = tubular maximum reabsorption of glucose

air Anesthesia was maintained by nembutal (5 mg/kg i v) when the goat showed signs of awakening Inulin 100 mg/kg was given i v followed by infusion of a solution in water of 0 6 % NaCl and 0 3 inulin at a rate of 12 5 ml/min for 30 min The infusion was continued with solutions of glucose in water in increasing concentrations (5 5 to 12%) and a constant concentration of inulin (0 4%) at a constant rate (0 2 ml/kg/min) Urine was collected through a bladder catheter at intervals of 15 min and venous blood was drawn at the 5th min of each clearance period

For the direct TmG determination i v infusion of a solution of 5 5% glucose and 0 35 inulin in water was given at a rate of 20 ml/min for 30 min and continued at a rate of 8 5 ml/min for an additional 90 min From the 60th min three clearance periods of 15 to 20 min were performed

Methods for determinations of inulin and glucose in blood and urine have been described recently (2) Serum calcium concentrations were determined by the method of Wilkinson (4)

RESULTS

The results of glucose titration studies demonstrate a well-defined tubular maximum reabsorption as measured in three goats (Fig 1) This maximum was reached at load/TmG < 1 5 There were no significant deviations between TmG determined by glucose titration and by the direct method (Table I) nor between two control determinations in any goat (Table II) TmG was not affected by widely spontaneous changes of the glomerular filtration rate in goat C Following injections of calcium and parathyroidectomy TmG decreased significantly but was raised by EDTA and parathyroid extract

DISCUSSION

Sherwood et al (3) beautifully demonstrated the parathyroid response to i v calcium and EDTA in goats as measured by radioimmunoassay Administration of calcium was immediately followed by decreased amounts of measurable parathyroid hormone in blood while EDTA had the opposite effect In this study calcium administration and parathyroidectomy led to a decreased tubular reabsorptive capacity for glucose while EDTA and parathyroid extract increased TmG The changes of TmG were not caused directly by alterations of the blood calcium concentrations as decreased as well as increased values of TmG were observed in both hypo and hypercalcemic states

These observations support the previous assumption of a positive relationship between the tubular reabsorptive capacity for glucose and the parathyroid function

ACKNOWLEDGEMENT

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Table II *Direct TmG determinations in two goats before and after induced alterations of the parathyroid function*

	Serum calcium (mg/100 ml)	BG (mg/ 100 ml)	GFR (ml/min)		TmG (mg/min)	
Goat C (67–138)						
Control	9	591	43 6	*42 8*	124 9	*128 7*
		640	40 9		121 6	
		694	43 9		139 6	
Control	9 3	583	63 9	*59 9*	140 8	*144 4*
		672	60 4		152 5	
		748	55 3		140 7	
		Mean TmG = 136 4 mg/min				
Calc um administr	10 9–10 7	759	37 8	*36 4*	107 4	
		790	36 5		109 3	
		858	*35 0*		*116 9*	
		Mean TmG = 111 2 mg/min				
Parathyroid extract	10 7–10 9	609	55 4	*51 6*	181 2	
		674	53 2		205 6	
		738	46 3		183 8	
		Mean TmG = 190 2 mg/min				
Goat D (67–148)						
Control	9 4–9 1	571	58 9	*54 9*	134 4	*128 3*
		630	55 5		126 5	
		677	49 2		124 0	
Control	*8 9*	*718*	*53 0*	*48 1*	*119 8*	*115 1*
		807	49 9		105 0	
		908	41 3		121 5	
		Mean TmG = 121 9 mg/min				
EDTA	6 9	584	6 1	57 4	149 1	
		698	56 8		170 6	
		740	53 3		144 4	
		Mean TmG = 154 7 mg/min				
Parathyroidectomy	6 8	596	42 5	*38 4*	101 1	
		626	37 0		95 0	
		652	35 6		101 1	
		Mean TmG = 99 1 mg/min				

REFERENCES

1 Halver B The effect of parathyroid hormone on the tubular reabsorption of glucose Acta med scand 179 4 7 1966

? — The diagnostic value of determination of tubular reabsorptive capacity for glucose in parathyro d disease Acta med scand 184 311 1968

3 Sherwood L M Potts J T Jr Care A D Mayer G P & Aurbach G D Evaluation by radioimmunoassay of factors controlling the secretion of parathyroid hormone Nature 209 52 1966

4 Wilkinson R H A micro-method for serum calcium and serum magnesium J cl n Path 10 1?6 1957

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THE DIAGNOSTIC VALUE OF DETERMINATION OF TUBULAR REABSORPTIVE CAPACITY FOR GLUCOSE IN PARATHYROID DISEASE

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Abstract Previous observations of changes of tubular reabsorptive capacity for glucose (TmG/GFR ratio) as a consequence of parathyroid disorder are supported by the results of a follow-up study of an additional forty five patients A distinct separation by TmG/GFR ratio exists in patients with hyper and hypoparathyroidism In five of thirty three patients with hyperparathyroidism (15) the values of TmG/GFR ratio were within mean ±. s.d of the values obtained in twenty four control patients Parathyroid extract increases and removal of parathyroid adenoma or suppression of the parathyroid function by i v calcium decreases the ratio

The value of a clinical application of this physiologic phenomenon is discussed The ratio might well point to the actual parathyroid function An increased ratio however does not illustrate whether a hyperparathyroidism is autonomous secondarily stimulated or due to secretion of a parathyroid hormone like substance And a decreased ratio does not exclude hyperparathyroidism since three patients with hyperparathyroidism due to water clear cell affection showed low values of the ratio Finally other factors than parathyroid hormone influence the reabsorptive capacity for glucose

In excluding coexisting hyperparathyroidism in hypercalcemic sarcoidosis and probably in separation of idiopathic hypercalcuria into a renal form with stimulated parathyroid function and an intestinal form with suppressed parathyroid function determination of the TmG/GFR ratio might be valuable

Recent observations indicate a positive correlation between tubular reabsorptive capacity for glucose and the parathyroid function (6 7 8) The purpose of this report is to elucidate this physiological phenomenon in an extended number of patients with parathyroid disorder and to discuss the value of a diagnostic test of the parathyroid function based on the effect of parathyroid hormone on the tubular reabsorptive capacity for glucose

MATERIAL AND METHODS

The material consists of seventy patients (Table I) Thirty seven patients had hyperparathyroidism verified during operation and subsequent histological examination of the removed glands Eight patients had hypoparathyroidism one idiopathic and seven postoperative substantiated by the finding of subnormal serum calcium concentrations for several years after strumectomy all seven patients were euthyroid One patient had severe uremia Twenty four patients with no signs of endocrine or metabolic disorder served as controls

Determination of glomerular filtration rate (GFR) and maximum tubular reabsorption of glucose (TmG) were carried out in all patients In seven of the hyperparathyroid patients the determination was repeated within 4 to 11 days after successful removal of parathyroid adenomas In seven patients (two controls four hypoparathyroid patients and one patient with severe uremia) the determination was repeated following i m administration of parathyroid extract, 150-200 I U twice a day for four days (Para Thor Mone® 100 U.S.P parathyroid units per ml Eli Lilly & Co) In one of the control patients the TmG determination was repeated after i v administration of calcium levulate 15 mg kg body weight during 10 hours

In five patients two of whom were in the postoperative state following removal of parathyroid adenomas the TmG determination was continued for 2 3 clearance periods following i v administration of 100 units of parathyroid extract

Observations in twenty five of the patients have been published previously (7) The results are recalculated according to a correction of the glucose inulin conversion factor (vide infra) and to the demand of a load TmG between 1 5 and 2 5 for all the hyperparathyroid and control patients

Some minor changes of methods previously described have taken place

Determinations of GFR and TmG were performed in the morning in all 70 patients In the fasting state venous blood was drawn for determination of blank values of inulin and fasting glucose concentration A minor meal (1 slices of white bread 1 cup of tea or milk) was allowed Adequate hydration was achieved by the administration of 1000 ml of water by mouth during one hour before the investigation Bladder catheterization was not performed Immediately before the investigation the patient micturated and on this urine blank values for glucose and inulin were obtained Infusions of a solution of 30% glucose and 0 3-0 6 % inulin in water (con

Table I *Serum calcium levels inulin clearances (GFR) and maximum tubular reabsorption of glucose (TmG) in patients with parathyroid disorder compared to control patients with no signs of endocrine disease*

Pat	Sex	Age (y)	Se-calcium (mg/100 ml)	GFR (ml/min)	TmG (mg/min)	TmG/GFR
Hyperparathyroid patients						
I A	♀	59	14 6	91 0	419 0	4 61
E H	♂	49	15 0	78 0	355 0	4 25
J T	o	45	11 3	22 8	72 9	3 20
K N	♂	54	10 9	132 5	420 7	3 19
A H J	♀	58	16 6	57 2	182 3	3 17
G M P	♀	57	14 7	30 0	93 4	3 11
P B	♂	45	11 3	129 2	389 8	2 99
I B	♀	53	15 7	29 1	86 0	2 96
K S	♀	60	10 9	99 1	284 7	2 87
S L	o	58	10 3	100 9	289 9	2 87
M E. J	♀	68	12 1	62 9	177 3	2 83
M R	♀	41	16 3	39 7	110 3	2 81
N K. C	♀	62	10)	101 7	283 7	2 77
E. K. J	♀	57	11 5	65 8	181 8	2 76
E E	♀	59	10 6	123 7	338 2	2 74
E. A	♂	66	11 7	63 3	171 6	2 72
K N	♀	54	15 9	36 0	96 7	2 68
S P	♀	55	11 7	69 4	184 7	2 67
B E. S	♀	23	11 8	46 7	124 4	2 66
M E.	♀	47	13 5	110 9	288 9	2 61
C S	♀	55	11 0	137 0	359 6	2 60
K. L	♀	54	10 4	121 3	310 7	2 56
R. A	♀	42	11 3	82 6	208 2	2 54
G I	♀	54	14 4	74 6	189 8	2 54
H M	o	57	10 8	102 4	258 8	2 53
E C	♂	53	15 5	60 1	150 6	2 52
J M	o	29	11 4	149 3	339 5	2 47
V C	♀	42	10 8	63 1	155 1	2 46
E. J	♂	49	9 6	73 4	177 0	2 42
V M	♀	64	11 5	81 2	179 5	2 21
G P	o	47	11 0	147 3	322 8	2 19
A R	♀	30	10 6	70 4	149 8	2 13
B S H	♀	24	10 5	99 9	193 7	1 94
Mean		50 6		83 4		2 78
Hypoparathyroid patients						
L O	♀	18	8 6	112 4	192 4	1 71
M K. H	♀	66	8 8	54 4	95 1	1 70
E J	♀	47	7 4	50 9	85 0	1 67
E T	♀	29	8 8	85 5	141 6	1 66
—	—	—	15 9[a]	58 8	49 7	0 85
P E. O	o	44	7 2	48 4	72 1	1 49
E B H	♀	51	11 3	31 5	29 0	0 93
M H	♀	42	9 2	118 1	103 8	0 88
I O	♀	59	9 6	83 6	72 1	0 86
Mean		44 5		71 5		1 31

Pat.	Sex	Age	Diagnosis	Se-calcium (mg/100 ml)	GFR (ml/min)	TmG (mg/min)	TmG/GFR
Control patients							
K. J	♀	54	Pyeloneph chr	9 5	50 5	128 0	2 52
J G	♂	61	Prol disci i v	9 6	112 6	277 2	2 49
O S	o	57	Spondylosis	9 2	136 0	310 7	2 29
S E.	♂	40	Cystis renes	9 3	21 7	49 1	2 27
F B	♂	48	Hernia	9 5	116 5	265 8	2 26
A A	o	44	Gastritis	9 5	113 0	247 4	2 19
V U	♂	53	Hernia	9 5	102 5	224 8	2 19
I M	♀	40	Pyeloneph chr	9 2	78 9	168 1	2 13
N N	o	50	Psychosis		99 6	205 3	2 06

Table I (*continued*)

Pat	Sex	Age	Diagnosis	Se-calcium (mg/100 ml)	GFR (ml/min)	TmG (mg/min)	TmG/GFR
H G	♀	24	Normal	9.6	98.7	201.8	2.03
G O	♀	60	Pyeloneph chr	9.4	25.9	51.7	2.01
L A H	♀	27	Neurosis hyst	9.5	101.9	203.6	2.00
W M	♂	18	Normal		156.0	312.7	2.00
E I N	♀	61	Tachycardia paroxystica	9.2	80.6	159.8	1.98
C S	♂	54	Resect ventric antea	9.9	146.3	289.0	1.98
H C	♀	58	Myoses nuchae	9.5	103.4	.05.4	1.96
A E H	♀	53	von Willebrandt	9.4	111.8	215.9	1.94
K S	♀	25	Anorex nervosa tractatae	9.8	102.8	198.1	1.94
P C	♂	74	Pyeloneph chr	9.7	24.6	48.1	1.91
H O	♂	42	Observatio	9.8	96.5	183.5	1.90
A M	♀	27	*Psychoneurosis*	9.5	112.3	214.3	1.90
A S	♀	32	Otosclerosis	9.0	104.4	195.0	1.89
N H	♂	4	Normal	9.3	128.7	240.9	1.87
I H	o	27	Neurosis anxiosa	9.4	143.0	266.0	1.86
Mean		43.9			98.7		2.06
Patients with wat r cl ar cell hyp rplas a adenoma							
A O	♂	52		14.1	83.6	151.3	1.81
—	—	—		15.6	73.2	116.0	1.58
M S	♀	52		11.3	91.6	156.0	1.71
—	—	—		11.7	94.5	146.8	1.56
R P	♂	56		14.8	36.5	42.4	1.17
Other patients							
T L	♀	54	Hyperparathyroidism	14.0	7.0	22.9	3.26
I R	o	28	Uremia	9.3[a]	7.7	12.4	1.63

[a] Hypercalcemia as a consequence of overtreatment with vitamin D

centration of inulin depending on body weight) was given in an antecubital vein at a rate of 12.5 ml/min for 1–20 min (depending on body weight). The infusion was continued with a solution of 1–20 glucose (depending on sex, age, weight and kidney function) and 0.2–0.4 inulin in water (depending on kidney function) at a rate of 8.5 ml/min throughout the investigation. The quoted doses of glucose ensured a load/TmG of more than 1.5 in all cases. All infusions were made with a constant infusion pump (10 gear finger pump, O. Dich, Hvidovre, Denmark). The patients micturated again 30 and 60 min after the start of the infusions to ensure that the bladder did not contain residual urine with high concentrations of glucose and inulin from the priming period. Following an equilibration period of 60 min, three consecutive clearance periods of 0 min were performed. Blood samples for determinations of inulin and glucose were drawn from the opposite antecubital vein at 3 min before the midpoint of each clearance period.

The patients received a total amount of liquid of 2000 ml within 3 hours, which ensured a mean diuresis of more than 6 ml/min in the clearance periods in most of the patients (3–… ml/min, mean 8.8 ml/min).

In one of the hypoparathyroid patients TmG was determined during glucose titration (that is, determination of tubular reabsorption of glucose under increasing filtered loads of glucose) as well as by the direct method. In one of the hyperparathyroid patients TmG was determined by glucose titration before and after extirpation of a parathyroid adenoma.

Glucose concentrations were determined in duplicate *by a modified glucose oxidase method*. *The modified* method used in this study has not been published previously (the method was worked out by S. E. Hansen, mag. scient., Institute of Biochemistry A, Juliane Mariesvej, Copenhagen, Denmark). A short outline is therefore given below.

Reagents

- *a* Enzyme buffer: 1. g of glucose oxidase (Boehringer GOD III), 12 mg of peroxidase (Sigma, crude), 600 ml of 1/15 *M* phosphate buffer (pH 7.0).
- *b* *o*-dianisidine: 0.5 g of *o*-dianisidine, 100 ml acetone.
- *c* Enzyme reagent: 150 parts of (*a*), 1 part of (*b*).
- *d* Perchloric acid (0.3 *N*): .5.6 ml perchloric acid 70% (density 1.7).
- *e* Standard solutions: standard 1 = 9.1 mg glucose, standard 2 = 18.. mg glucose, standard 3 = 27.3 mg glucose, standard 4 = 36.4 mg glucose,

to each of which is added 0.3 *N* perchloric acid to 100 ml. The four standard solutions correspond to 100, 00, 300 and 400 mg per cent glucose in serum.

Table II *Normal ranges of TmG/GFR ratio quoted from the literature*

	No of pat	Glucose determined in	TmG/GFR
Smith (16)	35	Arterial blood	2 72 ± 0 45 (1 s d)
Miller et al (12)	76	Arterial blood	2 81 ± 0 4ₐ
Goevaerts & Lambert (5)	45	Arterial blood	2 41 ± 0 35
Rieselbach et al (14)	24	Arterial blood	3 60 ± 0 36
Henningsen & Benveniste (9 10)	18	Venous blood	2 20 ± 0 30
This study	24	Venous blood	2 06 ± 0 19

[a] Nine control patients with endocrine disorders excluded

Procedure

1 0 ml of p rchloric acid and 0 1 ml serum is centrifuged for 10 min at 3000 rpm 0 2 ml of the supernatant is added to 5 ml reagent (*c*)

0 2 ml of a solution of urine in water 1 100 is added to 5 ml reagent (*c*)

0 2 ml of each standard solution is added to 5 ml reagent (*c*)

0 2 ml of 0 3 *N* perchloric acid is added to 5 ml reagent (*c*) (blank value)

The reaction is allowed to continue for 60 min The specimens are then read on a Beckman spectrophoto meter at 450 mμ

An estimate of analytical error was made by 24 deter minations of glucose concentrations in 18 sera with in creasing amounts of glucose (from 66 to 543 mg/100 ml) Standard deviations consistently increased from 1 4 to 4 1 mg/100 ml and variation coefficients gradually de creased from 2 4 to 0 8

The determination of inulin in blood and urine—also in duplicate—was performed according to the method of Bojesen (4) The recovery of 16 66 23 07 28 58 and 33 33 *mg/100 ml of inulin in serum from each patient* was 100 5 99 9 99 9 and 100 1 as determined in duplicate in 130 patients Standard deviations were 0 86 1 06 1 19 and 1 75 mg/100 ml The analytical error as measured by the mean standard deviation of 12 determina tions in duplicate of each of 6 sera containing 25 to 48 mg/100 ml of inulin was 0 3ₐ mg/100 ml (mean variation coefficient 0 9)

Since glucose interferes with the colorimetric deter mination of inulin estimation of a conversion factor was carried out for each patient To three sera from the fasting patient were added increasing amounts of glucose and the extinction for glucose inulin was determined In 120 determinations this conversion factor was 1 21 ± 0 27 (1 s.d) expressing the extinction of 100 mg of glucose as per cent of the extinction of 100 mg of inulin per 100 ml In urine the factor was found to be 0 69 The factor as determined in distilled water deproteinized distilled water and deproteinized urine were also found to be half the value for serum This difference could not be explained but had to be accepted because of its con stancy The relationship between glucose and inulin in serum in this study was about 15 1 and in urine 5 1 A *conversion* factor for serum of 1 21 and for urine of 0 69 indicated a correction of the measured con centrations of inulin in serum of 18 and in urine of only 3.5 Because the glucose concentrations in urine were equal in the three groups of patients to be com pared (hyperparathyroid patients mean 2795 mg/100 ml hypoparathyroid patients 2170 mg/100 ml and control patients 2340 mg/100 ml) the measured concentrations of inulin in urine were not corrected for the interference of glucose

RESULTS

The mean values of the TmG/GFR ratio as determined in the twenty four control patients were 2 06 ± 0 19 (1 s d) (Table I) These values are somewhat lower than those quoted from the literature (Table II) because in this study glucose was determined in venous blood instead of arterial blood (13)

The mean values of the TmG/GFR ratio in patients with hyperparathyroidism were 2 78 and in patients with hypoparathyroidism 1 36 (Table I)

Low values of TmG/GFR ratio were observed in three patients with clinically evident hyper parathyroidism An exploration of the neck re vealed primary water clear cell hyperplasia in two patients and a water-clear adenoma in one patient

The repeated determination of TmG/GFR ratio following i m administration of parathyroid ex tract for four days in four hypoprathyroid patients *and in three control patients with decreased* glomerular filtration rate showed increased values in all seven cases (Table III)

Continued TmG determination for 50–70 min following i v administration of parathyroid ex tract in three patients (A O M K H and N N) showed increased values of the TmG/GFR ratio The ratio of the two postoperative patients (N K C and T H L) however was not in creased by parathyroid extract administered intra venously (Table IV)

Table III *Determinations of TmG/GFR ratio in seven patients following i m administration of parathyroid extract (PTE) for three to four days and in one patient following calcium infusion*

Pat	Diagnosis	Se-calcium (mg/100 ml)	GFR (ml/min)	TmG (mg/min)	TmG/GFR
I R	Uremia	9 2	7 7	12 4	1 63
	+ PTE	9 9	9 3	23 4	2 50
G O	Uremia	9 4	25 9	51 7	2 01
	+ PTE	10 1	27 ?	66 7	2 45
K J	Uremia	9 5	50 6	128 0	2 52
	+ PTE	10 5	50 8	159 5	3 15
M K H	Hypoparathyr	8 8	54 4	95 1	1 70
	+ PTE	10 9	83 2	223 7	2 68
I O	Hypoparathyr	9 6	83 6	72 1	0 86
	+ PTE	14 5	76 3	115 5	1 54
L O	Hypoparathyr	8 6	112 4	192 4	1 71
	+ PTE	12 3	106 7	218 9	2 04
M H	Hypoparathyr	9 2	118 1	103 8	0 88
	+ PTE	11 3	123 3	166 4	1 36
Mean			64 7	93 6	1 62
+ PTE			68 1	139 0	2 25
diff			+ 5 3	+ 48 5	+ 38 9
J G	Prolaps disci i v	9 6	112 6	277 2	2 49
	+ calcium	11 7	107 7	234 8	2 18

Repeated determination of the TmG/GFR ratio within 4 to 11 days after successful removal of parathyroid adenomas in seven patients showed decreased values in all seven cases (Table V)

Table IV *Determinations of TmG/GFR ratio in five patients within one hour following i v administration of 200 units of parathyroid extract (PTE)*

Pat	Diagnosis	GFR (ml/min)	TmG (mg/min)	TmG/GFR
A O	Water-clear hyperplasia	73 -	116 0	1 58
	+ PTE	82 2	153 5	1 87
M K H	Hypoparathyroidism	33 0	56 0	1 70
	+ PTE	39 2	79 ?	2 0
N N	Control patient	99 6	05 3	06
	+ PTE	99 6	229 2	2 30
N K C	Postoperative hyperparathyroidism	113 7	279 5	2 46
	+ PTE	114 9	279 3	2 43
T L	Postoperative hyperparathyroidism	5 9	12 7	2 17
	+ PTE	6 1	12 2	2 00

Persisting hypercalcemia after removal of a parathyroid adenoma in this patient indicated the presence of an additional adenoma which was removed at a later operation

The TmG/GFR ratio decreased in one patient after administration of calcium (Table III)

TmG as determined directly did not deviate from TmG as determined during glucose titration (M H Fig 1) The glucose titration curves of two patients in hypoparathyroid states (M H and E H Fig 1) gave no direct evidence of an extended splay as a consequence of parathyroid hormone deficiency

DISCUSSION

Five of the hyperparathyroid patients (15) had a TmG/GFR ratio within 2 s D of that for the control patients There are several reasons to expect such an overlap primarily the dispersal of the ratio in the control patients secondly that probably other hormones exert some influence on the tubular transport of glucose (19) Certain drugs might influence the tubular reabsorption of glucose (a review is to be published) However even with a strictly hypothetical assumption that parathyroid hormone alone determines the TmG GFR ratio a certain overlap is to be expected Berson and Yalow (3) determined parathyroid hormone in plasma by radioimmunoassay

REFERENCES

1 Albright F Henneman P Benedict P H & Forbes A P Idiopathic hypercalcuria Proc roy Soc Med 46 1077 1953
2 Anderson J & Parsons V Maximal tubular reabsorptive rate for inorganic phosphate in hyperparathyroidism Brit med J 5391 1150 1964
3 Berson S A & Yalow R S Parathyroid hormone in plasma in adenomatous hyperparathyroidism uremia and bronchogenic carcinoma Science 154 907 1966
4 Bojesen E A method for determination of inulin in plasma and urine Acta med scand Suppl 266 275 1952
5 Govaerts P & Lambert I P Pathogénie du diabete renal Acta clin belg 4 341 1949
6 Halver B The effect of parathyroid hormone on the tubular reabsorption of glucose Acta med scand 179 427 1966
7 — The tubular transport of glucose as a measure of parathyroid function Acta med scand 181 209 1967
8 — Relation of tubular maximum reabsorption of glucose and parathyroid function in goats Acta med scand 184 307 1968
9 Henningsen P & Benveniste D Effect of tolbutamide on renal glucose reabsorption Diabetes 15 90 1966
10 — Effect of hydrochlorothiazide and chlorthalidon on renal reabsorption of glucose Scand J clin Lab Invest 17 388 1965
11 Hornum I Transbøl I Hahnemann S & Halver B Idiopathic hyperabsorption hypercalcemia in the adult An endocrine and metabolic study 5 symposium européen sur les tissus calcifiés Bordeaux 1967
12 Miller J H McDonald R K & Shock N W Age changes in the maximal rate of renal tubular reabsorption of glucose J Geront 7 196 1952
13 Pitesky I Last J H & Epperson E B Approximation of the tubular maximum for reabsorption of glucose using venous blood for plasma glucose levels Amer J Physiol 165 407 1951
14 Rieselbach R E Shankel S W Slatopolski E Lubowithz H & Bricker N S Glucose titration studies in patients with chronic progressive renal disease J clin Invest 46 157 1967
15 Sherwood L M Potts J T Jr Care A D Mayer G P & Aurbach G D Evaluation by radioimmunoassay of factors controlling the secretion of parathyroid hormone Nature 209 52 1966
16 Smith H W Lectures on the kidney University of Kansas Lawrence 1943
17 Tashjian A H Jr Levine L & Munson P L Immunochemical identification of parathyroid hormone in non parathyroid neoplasms associated with hypercalcemia J exp Med 119 467 1964
18 Transbøl I & Halver B Relation of renal glycosuria and parathyroid function in hypercalcemic sarcoidosis J clin Endocr 27 1193 1967
19 Wesson L G Jr Hormonal influences on renal function Ann Rev Med 12 77 1961

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CELLULAR HYPERSENSITIVITY IN SJÖGREN S SYNDROME

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Abstract An in vitro method based upon the specific action of human salivary gland extract upon the migration of leucocytes is presented. The results obtained with this method ind cate that a certain state of cellular hyper sens tivity directed against the salivary glands is present in two-thirds of the patients with Sjogren s syndrome It appears that there is no correlation between the presence of circulating antibodies and the presence of cellular hypersensitivity Furthermore it is not possible from the presented data to conclude whether the cellular hyper sensitiv ty has a closer relationship to the pathogenesis than the presence of circulating antibodies

In 1958 Jones demonstrated the presence of a precipitating antibody in three patients with Sjogren s syndrome As a result of this and the presence of increased gamma globulin in sera from 50% of the patients with the syndrome he pointed out that the aetiology of Sjogren s syndrome might be analogous to that demonstrated in Hashimoto s thyroiditis i e an autoimmune mechanism The phenomenon described was non specific (20) and so was the complement fixing antibody demonstrated by Mosbech and Østergaard Kristensen (24) Anderson et al (1) and Block et al (6) In 1964 Bertram and Hafberg (4) described a phenomenon representing a specific anti body to an antigen in the cytoplasm in the epithelium of the salivary du ts No antibodies to components in the acini were found These findings have been confirmed by Feltkamp and van Rossum (12) An attempt to correlate the characteristic histological findings described by Godwin (14) Morgan (22) Cardell and Curling (7) and Heusler (18) with the presence of circulating antibodies directed against the salivary glands has until now failed (5)

Consequently the interest has been focused upon cellular hypersensitivity as a possible pathogenetic factor concerning the changes of the salivary glands in Sjogren s syndrome

This assumption is directly supported by the infiltration of lymphocytes and other mononuclear cells in the salivary glands and indirectly by the demonstration of organ specific cellular hyper sensitivity in other diseases in which auto immune mechanisms are believed to be involved i e Hashimoto s thyroiditis (31 34) colitis ulcerosa (2 25) and glomerulonephritis (3) The purpose of the present study has been to demonstrate a state of cellular hypersensitivity directed against con stituents of the human salivary glands by means of an in vitro technique This method originally described by Rich and Lewis (26) and later applied to man by Søborg and Bendixen (29) seems to be able to demonstrate cellular hypersensitivity in vitro

MATERIAL AND METHODS

The material consisted of 14 patients with Sjogren s syn drome diagnosed from the presence of keratoconjuncti itis sicca xerostomia changes in the oral mucosa radiographical changes in the parenchyma of the sali ary glands, histologically demonstrable chronic sialoadenitis of the benign lymphoepithelial lesion type associated with systemic symptoms pertaining to the syndrome especially rheumatoid arthritis It was considered sufficient if two in dependent symptoms were present Eleven persons without any evidence of disease in the sali ary gland or rheumatoid arthritis were used as controls

The average age of the patient group was 58 ranging from 40–74 years The control group consisted of persons with an average age of 44 ranging from 19–69 years

Leucocyte migration studies and determination of anti bodies were performed at the same time in each in div dual

Leucocyte migration studies

The technique has been described in detail in a previous paper (29) White blood cells were obtained from the

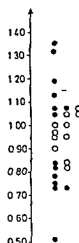


Fig 1 The distribution of migration indices in 14 patients *with Sjogrens syndrome and in 11 controls* • *patient* with Sjogrens syndrome O control

peripheral blood and after thorough washin, the cells were placed in capillary tubes then put into tissue culture chambers and tissue culture medium was added An extract prepared from human salivary glands according to *the principle described by Goudie et al (15)* was used as an antigen One hundred μl of this extract was added to half of the cell cultures

The cell migration areas were measured after 24 hours and the average migration area of the antigen containing cultures *M* was related to the average migration area of the control cultures *M* and expressed as follows viz *M /M* – migration index.

Thus the numerical value of the migration index expresses whether th migration has been stimulated or inhibited by the antigen An index greater than 1 00 means stimulation and less than 1 00 inhibition of the cell migration

Demonstration of circulating antibodies

Antibodies to the cytoplasm in the salivary duct epithelium were demonstrated by means of Coons and Kaplans (10) immunofluorescence technique on unfixed sections (4-6 μ thick) of normal human salivary gland tissue as described by Bertram and Halberg (4)

RESULTS

Fig 1 shows the distribution of the migration indices of 14 patients with Sjogrens syndrome and 11 control persons The observations from the patients with Sjogrens syndrome seem to fall in three categories one characterized by inhibition of the migration another by stimulation and a third showing neither inhibition nor stimulation When the indices of the Sjogrens syndrome patients are regarded as a group the mean value is 0 95 and the standard deviation 0 25 while the mean value of the controls is nearly the same *i e 0 97 but with a standard deviation of 0 09* This difference in the standard deviation means that the observations from the patients and the controls form two significantly different populations ($f = 7\ 69$ $P < 0\ 01$) Table I shows the findings of circulating antibodies and the corresponding migration indices of the patients with Sjogrens syndrome

It appears that there is no correlation between the degree of cellular hypersensitivity and the presence of circulating antibody There were no circulating antibodies in the sera from the control persons

DISCUSSION

The alteration of the migration capacity in vitro of immunocompetent cells upon contact with specific antigen is a well established parameter of cellular hypersensitivity The majority of experiments using different antigens and various sources of immunocompetent cells have demonstrated that the action of antigen resulted in an inhibition of the cell migration (8 9 11 13 17 19 23 26 27 30 33) well correlated to the delayed intracutaneous reaction On the other hand a few experiments have indicated that a stimulation of the migration can also be observed (16 21 28 32) The inhibition and stimulation of the migration

Table I *The occurrence of circulating antibodies (cytoplasmic fluorescence) and cellular hypersensitivity (migration index) to salivary gland components in 14 patients with Sjogrens syndrome*

Pat no	Cytoplasmic fluorescence	Migration index
1	+	0 52
2	–	0 70
3	+ +	0 73
4	–	0 75
5	+	0 78
6	+	0 82
7	+	0 83
8	+ +	1 05
9	+	1 06
10	+	1 06
11	–	1 13
12	+	1 19
13	+	1 32
14	+	1 35

se m to be both closely related and to be specific expressions of cellular hypersensitivity in vitro This interrelationship has recently been analysed by Søborg (32) in experiments with brucella hyper sensitivity The conclusion of these experiments was that the resulting action of antigen upon the cell migration depended upon at least two factors 1 the degree of sensitivity of the cell donor and 2 the antigen concentration At a fixed relatively low antigen concentration a high sensitivity of the cells resulted in an inhibition a low sensitivity in a stimulation and an intermediary degree of sensitivity gave *neither inhibition nor stimulation*

If on the other hand the sensitivity of the cells was fixed then high antigen concentrations gave inhibition and low concentrations stimulation while intermediate concentrations apparently left the migration uninfluenced by the antigen If the observations were regarded as a group without any subdivision according to sensitivity the highest antigen concentration always resulted in an in hibition while the distribution of indices of the lower concentrations covered the whole range from stimulation to inhibition The non sensitive controls were all grouped around an index of 1 00 with minor fluctuations at the different antigen levels

The present results show that the migration indices of the patients with Sjogren s syndrome are spread within a wide area indicating a stimulation as well as an inhibition of leucocyte migration upon contact with antigen from the salivary glands The distribution of the indices is similar to the distribution observed in the above mentioned brucella experiments at one of the submaximal antigen concentrations This similarity suggests that the cells originate from persons with varying degrees of cellular hypersensitivity to an extract of salivary glands

Because of five observations within the normal range it is impossible to say whether the total population investigated is in a state of cellular hypersensitivity On the other hand the data pre sented allow the conclusion that at least two-thirds of the patients show a certain degree of cellular hypersensitivity as indicated by the in vitro test Whether this percentage is the true incidence of cellular hypersensitivity in Sjogren s syndrome pa tients is impossible to say at the moment. The presence of stimulation as well as inhibition in dicates in analogy with the brucella experiments that the antigen concentration applied is too low If higher antigen concentrations had been used a more precise quantitation of the cellular hyper sensitivity could probably be obtained From the data presented a certain estimate can be made of the degree of sensitivity The patients with an inhibition of the cell migration may be considered more sensitized than the patients with indices indicating a stimulation As regards the observa tions within the normal range it is impossible to predict whether these represent an intermediary degree of sensitivity or no sensitivity at all

As previously mentioned there is no correla tion between the degree of cellular hypersensitivity and the presence of circulating antibody

The conclusion must be that humoral as well as cellular hypersensitivity is present in Sjogren s syndrome directed against components in human salivary glands

The results show that a registration of both types of immunological reactions is necessary in order to get further information concerning the pathological mechanism involved in the changes of the salivary glands in Sjogren s syndrome

Whether the cellular hypersensitivity is more closely related to the pathogenesis of the benign lymphoepithelial lesion in Sjogren s syndrome than is the presence of circulating antibodies has not been clarified by the experiments here presented But it must still be kept in mind that immuno logical reactions and phenomena are related to many diseases without telling much about the pathogenesis of the diseases

REFERENCES

1 Anderson, J R Gray K G Beck, J S & Kinnear W F Lancet ? 456 1961
2 Bendixen G Scand J Gastroent . .14 1967
3 Bendixen G Acta med scand In print
4 Bertram U & Halberg, P Acta allerg (Kbh) 19 458 1964
5 Bertram U Acta odont scand Suppl. 49 1967
6 Bloch K. J Buchnan W W Wohl M J & Bunim J J Medicine 44 187 1965
7 Cardell B S & Gurling, K J J Path Bact 68 137 1954
8 Carpenter R. R J Immunol 91 803 1963
9 Carpenter R. R & Brandriss M W J exp Med 1 0 1231 1964
10 Coons A. H & Kaplan M H J exp med Sci 91 1 1950
11 David J R., Al Askari S Lawrence H S & Thomas L J Immunol 93 ?64 1964

12 Feltkamp T E W & van Rassum A L Clin exp Immunol 3 1 1968
13 George M & Vaughan J Proc Soc exp Biol (N Y) 111 514 196
14 Godwin J T Cancer (Philad) 5 1089 195_
15 Goudie R B Anderson J R Gray K G Clark D H Murray J P C & McNicol G P Lancet 2 976 1957
16 Hall H E & Scherago M Amer Rev Tuberc 75 807 1957
17 Heilman D H Howard D H & Carp nter C M J exp Med 107 319 1958
18 Heusler A Pract oto rhino laryng (Basel) 23 73 1961
19 Johnson R W & Scherago M Amer Rev resp Dis 81 96 1960
20 Jones B R Lancet 2 773 1958
21 Juhasz Schaffer A Z Immun Forsch 56 25 19_8
22 Morgan W S New Engl J Med 251 5 1954
23 Morn J K J exp Med 64 355 1936
24 Mosbech J & Østergård Kristensen H P Ugeskr Læg 12? 953 1960
25 Perlmann P & Broberger O J exp Med 117 717 1963
26 Rich A R & Lewis M R Bull Johns Hopk Hosp 50 115 193?
27 Svejcar J & Johanovský J Z Immun Forsch 1_? 398 1961
28 — Z Immun Forsch 131 301 1966
29 Søborg M & Bendixen G Acta med scand 181 _47 1967
30 Søborg M Acta med Scand 18_ 167 1967
31 Søborg M & Halberg P Acta med scand 183 101 1968
32 Søborg M Acta med scand In print
33 Thor D E Science 157 1567 1967
34 Wolf Jurgensen P & Halberg P Acta Allerg (Kbh) _0 438 1965

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THE CELLULAR CONTENT IN NON TIMED SPECIMENS OF URINE

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Abstract The present investigation is a report on the cellular content in non timed midstream specimens of urine from 643 ambulant males and females and was performed in connection with an examination of blood pressure in the population of B rgen Norway Special regard was paid to the influence of the specific gravity of the urine and to bacteriuria (including bacterial conta mination < 10 bacteria/ml)

Specimens from males and females w thout previous urinary tract disorders contained ⩽ 7 8 non-disrupted erythrocytes/mm in approximately 95 of the cases and this value was therefore assumed to be a suitable *upper limit for normal counts in routine work*. The number of erythrocytes was found to be lower in speci mens at specif c gravities below about 1 010 than above hypotonic haemolysis being the probable cause of the difference

Correspond ngly ⩽ 13 leucocytes + non-squamous epi thelial cells/mm were found in approximately 95% of the sterile specimens from males and females without previous urinary tract disorders Sterile specimens from females did not contain more cells than specimens from males but specimens with bacterial contamination con tained slightly greater numbers of cells than sterile speci mens In males greater numbers of leucocytes + non squamous epithelial cells were recorded in specimens at h gh than at lower specific gravities in females high cell counts were most frequently observed at the lower specific grav t es Less than 10 leucocytes + non-squamous epi thelial cells were recorded in about half of the specimens from individuals with bacteriuria A def nite borderline between normal and pathological counts can therefore not be established

In females bacteriuria at the level 10 –10⁵ bacteria/ml was most often observed in specimens at specific gravi- ties ⩾ 1 019 An inhibitory effect on bacterial growth in urine of h gh spec f c gra ity is suggested.

The distinction between normal and pathologi cally increased numbers of cells in urine is im portant in clinical work Previous investigations indicate that ⩽ 5 erythrocytes/mm³ are present in urine from most healthy adults (23 27) and children (1 21) and < 10 white cells/mm³ in 76–100° of specimens from healthy adults (3 7) and children (14 21 24 25) These values have therefore been regarded as the normal up- per limits Higher normal limits for white cells have also been suggested 12 cells/mm³ (17) 20 cells/mm³ (1) 25 and 50 cells/mm³ in newborn boys and girls resp ctively (16 19) 5 and 50 cells/mm³ in males and f^males respectively (4) Various selections of materials and various methods of collecting and examining specimens may account for the differences

In the case of ambulant patients non timed specimens of urine are most easily obtainable and a knowledge of the limits of normal and pathological cellular content in such spe^imens is therefore a point of practical importance The object of the present investigation was to study the cellular content per mm³ in non timed mid stream specimens of urine from ambulant persons without and with previous or present urinary tract disorders Special attention was paid to factors that might influence the cell counts such as urinary concentration (specific gravity) and the presence of bacteria (contamination or true bac t riuria)

MATERIAL AND METHODS

An epidemiological invesugation of blood pressure in the population of Berg n provided an opportunity of study ing the cellular content in urine specimens from ambulant persons

Histories of prev ous and present symptoms and diseases were obtained by a nurse according to a question naire and were completed in connection with clinical examination by an internist Special regard was paid to card o vascular and urinary tract disorders (cystitis pye lonephr tis urolithiasis, dysuria pollakisuria, proteinuria includ ng prote nuria during pregnancy Routine examina tion of genitals was not included (^6)

Midstream specimens of urine were collected after in

Table I *Age distribution of the material (281 males 362 females)*

	Males		Females	
Age groups (y)	(No)	( )	(No)	( )
23–32	48	17 1	65	17 9
33–42	48	17 1	66	18 2
43–52	72	25 6	80	22 1
53–62	69	24 6	84	23 2
63–72	44	15 7	67	18 5

struction by an experienced nurse Cleaning of genitals was performed before voiding by drawing each of three sterile swabs soaked with water and soap once over the urethral orifice males after retraction of preputium non menstruating females from front to back after having spread the labia with one hand Females passed their urine in standing position, holding the labia apart The procedure was not supervised unless the nurse judged it necessary or the person asked for assistance

Protein ugar and bacteriological examinations of the urine were carried out haematuria examinations not being made unless Heller s test was positive One portion of the urine reserved for bacteriological examination was immediately placed in a refrigerator usually being cultured the following day Identification of bacteria was performed according to standard technique and quanti tative culture by spreading 0 5 ml of a 1 100 and 1 10 000 dilution of urine on blood agar Colony counts were made 24 hours later If ⩾10 bacteria/ml had been recorded a second specimen was obtained usually after 2 weeks occasionally later

During a part of this study the numbers of non disrupted erythrocytes disrupted or partly haemolysed erythrocytes leucocytes and non-squamous epithelial cells per mm urine were counted by the author Counts were made on uncentrifuged and unstained specimens in 1 mm in an improved Neubauer haemocytometer Specific gravity and pH were determined Nothing except the sex was known about the persons whose urine was examined until the completion of the study

The present material consisted of 643 consecutive specimens (from 281 males and 362 females) submitted for the first bacteriological examination a second spe cimen from 42 cases which on the first examination had bacteriuria (⩾10 bacteria ml) and from 16 cases which on the first count had ⩾21 red or white cells/mm The age distribution of the individuals is shown in Table I

In the analysis of the material a heterogenous group of 43 males and 25 females with sterile or bacterially contaminated urine (<10⁴ bacteria/ml) but with various conditions that might influence the cellular content in urine was excluded because the subgroups became too small to permit conclusions to be drawn This group comprised individuals with proteinuria, glucosuria dia betes cardio-vascular disorders hypertension (systolic blood pressure ⩾200 mm Hg diastolic ⩾100 mm Hg) chronic bronchitis, and those taking drugs continuously The rest of the material was divided into three groups

Group I

One hundred and sixty three males and 112 females who had never had symptoms or signs of disorders related to the urinary tract nor diseases that might influence the urinary tract Their urine did not contain protein or sugar and was sterile or contained <10 bacteria/ml (regarded as contamination)

Group II

Seventy-one males and 187 females giving a history of previous symptoms or signs related to the urinary tract but no other diseases that might be assumed to influence the urinary tract As in group I urinalysis revealed no pathological findings On the basis of the available in formation further differentiation was not justifiable

Group III

Four males and 38 females with bacteriuria (⩾10 bac teria/ml) on the first examination Other pathological conditions might, or might not be present

RESULTS

Group I

The specific gravities of urines from males ranged from 1 001 to 1 028 from females from 1 003 to 1 029 The percentage distribution of specific gravities is shown in Table II A bimodal distribu tion apparently occurred in females with a greater number of specimens at specific gravities ⩽1 010 than in males

The pH values of urines from males ranged from 5 0 to 8 5 in females from 4 8 to 8 4 The percentage distribution of pH values is shown in Table III

The percentage distribution of erythrocytes

Table II *Percentage distribution of specific gravities in non timed specimens of urines from 163 males and 112 females without previous or present urinary tract disorders (group I)*

Spec grav	⩽1 005	1 006–1 010	1 011–1 015	1 016–1 020	1 021–1 025	⩾1 026
Males ( )	4 9	8 6	13 5	35 0	26 4	11 7
Females ( )	8 0	23 2	17 0	24 1	20 5	7 1

Table III *Percentage distribution of pH values in non timed specimens of urines from 163 males and 112 females without previous or present urinary tract disorders (group I)*

pH	≤5	5 1–5 5	5 6–6 0	6 1–6 5	6 6–7 0	7 1–7 5	≥7 6
Males (%)	1 2	3 1	44 2	28 8	18 4	3 1	1 2
Females ( )	5 4	8 9	45 5	2? 3	14 3	2 7	0 9

(non disrupted disrupted and total) and leucocytes non squamous epithelial cells and white cells combined is shown in Table IV

In routine work an upper limit of cell content comprising 95% of the specimens may be regarded as a suitable normal limit Specimens from 96 9% of the males and 94 6 of the females contained ≤7 non-disrupted erythrocytes/mm³ When disrupted and partly haemolysed erythrocytes were included <12 and <11 erythrocytes/mm³ were found in specimens from 95 1% of males and 95 5 of females respectively

The upper limit of a normal white cell count should be based on the cell content in sterile specimens since specimens with bacterial contamination (diphtheroids micrococci <10⁴ coli or proteus/ml) contained greater numbers of white cells than sterile specimens from females while sterile specimens from females had approximately the same cell content as specimens from males (Table V) Sterile specimens from 94 7 of males and 95 1% of females contained ≤13 leucocytes+non squamous epithelial cells/mm³

Bacterial contamination was noted in 12 specimens (7 4%) from males and in 51 specimens (45 5) from females About half of the specimens containing >20 white cells/mm³ were sterile

Scatter diagrams revealed that the numbers of disrupted erythrocytes were unrelated to the specific gravity in both sexes Smaller numbers of non-disrupted erythrocytes were noted in specimens at specific gravities ≤1 010 being more pronounced in specimens from males (Fig 1) than from females (Fig 2)

Scatter diagrams also revealed that specimens from males contained greater numbers of cells separately recorded as leucocytes and as non squamous epithelial cells at high specific gravities than at low while in females the numbers of these cells were unrelated to the specific gravities However leucocytes are not always easily distinguished from non squamous epithelial cells in unstained specimens The relation between

Table IV *Percentage distribution of various kinds of cells in non timed specimens of urines from 163 males and 112 females (group I)*

Cells/mm³		0	1	2	3	4	5	6	7	8	9	10	11–20	>21
Erythrocytes non disrupted	Males	39 3	27 0	13 5	6 1	3 7	2 5	1 8	1 2	0 6	0 6	0 6	0 6	2 5
	Females	44 6	5 0	12 5	6 3	3 6	2 7	0	0 9	0 9	0	0	1 8	1 8
Erythrocytes disrupted	Males	13 5	?6 4	19 0	19 0	9	2 5	4 3	3 1	0 6	0 6	0	1 8	0
	Females	16 1	4 1	17 0	1 5	6 3	10 7	7	1 8	3 6	0	2 7	1 8	0 9
Erythrocytes total	Males	7 4	16 6	16 0	17 8	11 7	11 0	3 7	1 2	2 5	1 2	3 7	4 2	3 1
	Females	8 0	20 5	17 0	11 6	8 9	4 5	8 0	3 6	2 7	7	4 5	5 4	2 7
Leucocytes	Males	37 4	31 3	13 5	3 7	5	1 8	1 8	1 8	0	0	1 2	3 7	1 2
	Females	35 7	17 9	10 7	8 9	4 5	2 7	1 8	4 5	0 9	0 9	0	4 5	7 1
Non-squamous epithelial cells	Males	48 5	24 5	12 3	8 0	3 1	1	1 2	0	0 6	0	0	0 6	0
	Females	48 2	22 3	14 3	6 3	3 6	0 9	3 6	0	0	0	0	0 9	0
Leucocytes and non squamous epithelial cells	Males	24 5	25 2	17 8	11 7	4 3	1 8	2 5	0 6	1 2	1 8	1 8	4 3	2 5
	Females	24 1	18 8	10 7	9 8	9 8	2 7	0	0 9	2 7	4 5	3 6	5 4	7 1

Table V *Comparison of the percentage distribution of leucocytes + non squamous epithelial cells/mm³ in sterile and bacterially contaminated (<10⁴ bact /ml) urine from males and females without (group I) and with (group II) previous urinary tract disorders*

	Males				Females			
	Sterile		Contam		Sterile		Contam	
Group	I	II	I	II	I	II	I	II
No of persons	151	62	12	9	61	115	51	72
Cells mm³								
0–5	84 8	82 3	91 7	77 7	82 0	86 1	68 6	56 9
6–10	8 6	9 7	0	11 1	8 2	6 1	15 7	[illegible]
11–20	4 0	3 2	8 3	0	6 6	3 5	3 9	8 3
≥21	2 7	4 8	0	11 1	3 3	4 3	11 8	12 5

these cells combined and the specific gravity of the urine is shown for males in Fig 3 and for females in Fig 4 At specific gravities <1 010 none of the specimens from males (22 in all) but about half of the specimens from females (17 out of 35) contained >3 white cells/mm³ At higher specific gravities >3 cells/mm³ were found in about a fourth (34 out of 141) of the specimens from males and about a third (25 out of 77) of the specimens from females Of the specimens containing >3 white cells/mm³ only three specimens from males but about half of the sp cimens from females revealed bacterial ontamination Sterile and bacterially contami nated specimens with various cell content were intermingled at all specific gravities In specimens from males the higher white cell counts were consequently observed only at the higher specific gravities in specimens from females most fre quently at low specific gravities

Scatter diagrams revealed no relation between the numbers of red or white cells and the pH values of the urines

Comparison of the numbers of non disrupted erythrocytes and leucocytes + non squamous epi thelial cells in the age groups 23–47 and 48–72 years revealed only minor differences (Table VI)

Group II

Comparison of the percentage distribution of cells in this group with the distribution of cells in group I revealed good agreement (Table VII)

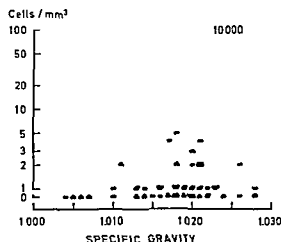


Fig 1 Relation between erythrocytes (non-disrupted) per mm³ and the specific gravity of the urine from 163 healthy males (group I)

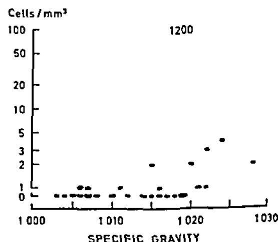


Fig ? Relation between erythrocytes (non-disrupted) per mm and the specific gravity of the urine from 112 healthy females (group I)

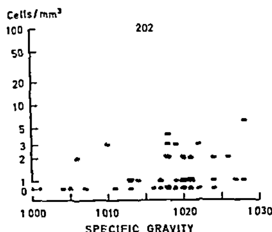


Fig 3 Relation between leucocytes + non-squamous epithelial cells/mm and the specific gravity of the urine from 163 healthy males (group I)

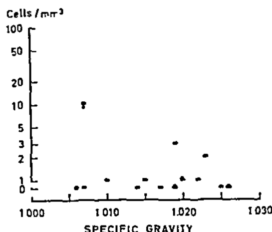


Fig 4 Relation between leucocytes + non squamous epithelial cells/mm and the specific gravity of the urine from 112 healthy females (group I)

Bacterial contamination was noted in specim n from 12 7% of the males and 38 5% of the females The distribution of leucocytes + non squamous epithelial cells in sterile specimens from males and femal~s respectively in group I and group II agreed fairly well while bacterially contaminated specimens from females contained greater numbers of white cells in group II than in group I (Table V) The numb~r of contaminated specimens from males was too low to allow valid comparison of th~ groups

Group III

On the first examination four specimens from males contained $\geq 10^4$ bacteria/ml two of them > 10 bacteria/ml E coli was cultured in three specimens enterococci in one specimen The numbers of leucocytes + non squamou epithelial cells in these specimens were found to be 12 26 43 and 532 cells/mm³ respectively Similar results were obtained on examination of the second specimen

Thirty-eight sp cimens from females contained $> 10^4$ bacteria/ml (Table VIII) in twelve of these cases a negative history of urinary tract disorders had been obtained A negative history was also obtained in five of the 19 cases containing > 10 coli/ml in both specimens E coli or species from the coliform group of bacteria were found in 35 cases in three cases α or β haemolytic streptococci or staphylococci were recorded On the second examination some weeks later five specimens which on the first examination had been infected with coli and the three specimens which had b~en infected with cocci were either sterile or contained $< 10^4$ bacteria/

Table VI *Percentage distribution of erythrocytes (non-disrupted) and leucocytes + non squamous epithelial cells mm in urine in the age groups 22–47 and 48–72 years (group I)*

	Erythrocytes				Leucocytes + non-squam epithel			
	Males		Females		Males		Females	
Age group	23–47	48–7	23–47	48–7	23–47	48 7	23–47	48–7
No of perso s	95	68	56	56	95	68	56	56
Cells mm								
0–5	93 7	89 7	94 6	94 6	85 3	85 3	78 6	73 2
6–10	4 [illegible]	5 9	1 8	1 8	5 3	11 8	10 7	1[illegible] 5
11 0	0	1 5	1 8	1 8	6 3	1 5	1 8	8 9
21	[illegible] 1	[illegible] 9	1 8	1 8	3 2	1 5	8 9	5 4

Table VII *Percentage distribution of leucocytes + non squamous epithelial cells/mm³ in urine of group II and group I*

	Males		Females	
Group	I	II	I	II
No of persons	163	71	11.	187
Cells mm³				
0–5	85 3	81 6	75 9	74 9
6–10	8 0	9 9	11 6	12 3
11–20	4 3	2 8	5 4	5 4
≥21	2 5	5 6	7 1	7 5

ml With the exception of the three specimens infected with cocci (contamination?) and one in fected with coli all specim ns with ≥10⁴ coli/ml were obtained from females older than 45 years

One special factor concerning bacteriuria and the specific gravity of the urine was noted In specimens with 10⁴ – < 10⁵ coli or coliform bac teria/ml 13 out of 15 specimens had specific gravities ≥1 019 while in specimens with ≥10 coli or coliform bacteria/ml 17 out of 50 speci mens had specific gravities ≥1 019 (Table VIII)

Table VIII *The number of leucocytes + non squamous epithelial cells/mm³ and the corresponding specific gravity of the urine at various levels of bacteriuria (coli or cocci) in 38 females*

x indicates specimens infected with cocci

	First count		Second count		
Bact /ml	Cells/mm³	Spec grav	Bact /ml	Cells/mm³	Spec grav
10⁴–< 10⁵	5	1 013	< 10⁴	12	1 015
	5	1 0?2	10⁴–< 10⁵	11	1 020
	6	1 023		36	1 021
	38	1 022		58	1 022
	4	1 011	10 – < 10	10	1 017
	55	1 024		15	1 006
	79	1 019		0	1 014
	80	1 023		32	1 0 2
	6040	1 020		81	1 0?3
	9	1 021	10	8	1 014
10⁵–< 10⁶	3x	1 0?3	< 10⁴	2	1 015
	5x	1 023		.	1 016
	7	1 028		30	1 030
	3	1 0?0	10 – < 10⁵	3	1 019
		1 007	10⁵–< 10⁶	17	1 004
	4	1 017		9	1 0.2
	16	1 023		33	1 015
	4	1 005	10⁶	34	1 011
	28	1 006		9?	1 007
≥ 10⁶	.x	1 004	< 10⁴	0	1 008
	8	1 019		38	1 016
	14	1 028		27	1 0.7
	22	1 013		10	1 017
	8	1 021	10 – < 10⁵	6	1 023
	6	1 0.0	10⁵–< 10⁶	5	1 017
	51	1 016		20	1 022
	93	1 006		81	1 005
	1	1 005	10	0	1 008
	3	1 016		14	1 010
	4	1 009		3	1 003
	5	1 015		3	1 006
	8	1 018		6	1 016
	15	1 014		?1	1 011
	.0	1 014		1	1 017
	38	1 019		64	1 023
	62	1 018		7	1 012
	63	1 0?3		802	1 0 1
	149	1 0?2		79	1 023

The majority of the pH values in the specimens were 5 6–6 5 three specimens had pH > 7 There was no relation between bacteria/ml and the pH of the urine

Ten or less leucocytes + non squamous epithelial cells/mm³ were recorded in almost half of the specimens (Table VIII) Scatter diagrams revealed no relationship between cells/mm³ and bacteria/ml or between cells/mm³ and specific gravity of urine

DISCUSSION

In the present material ⩽ 7 non disrupted erythrocytes/mm³ were recorded in about 95° of non timed midstream specimens of urine from males and females without previous urinary tract disorders (group I) The smaller numbers of non disrupted erythrocytes in specimens of specific gravity ⩽ 1 010 (Figs 1 2) were most probably due to hypotonic haemolysis (11) Such specimens may be unsuitable for quantitative estimation of non-disrupted erythrocytes when these are excluded < 8 non disrupted erythrocytes/mm³ were found in about 95 % of the specimens from males and females Reismann (23) assumed that ⩾ 7 erythrocytes/mm³ might be regarded as pathologically increased

Though 7–8 non-disrupted erythrocytes/mm³ may be a suitable upper limit for normal counts in routine work the variables of the method must be kept in mind The random variability of haemocytometer counts implies that if e g 8 cells/mm³ is an estimate of the true number succeeding counts of new samples from the same specimen may vary approximately between 3 and 16 cells in 95 of the occurrences (5 10) Moreover the variable shape of erythrocytes in urine (9) results in considerable observer differences in distinguishing between cells recorded as non disrupted erythrocytes and erythrocytes with a leaky or disrupted appearance In cases of doubt repeated examinations of the same specimen and of new specimens are necessary In group I *⩾ 21 erythrocytes (total)/mm³* were recorded in specimens from five males and three females some of these counts being very high In a second specimen all but one contained < 8 erythrocytes/mm³ Further clinical examination may elucidate the significance of the higher counts but this is beyond the scope of this presentation

In agreement with previous reports (6 28) greater numbers of non disrupted than leaky or disrupted erythrocytes were found in urines from *individuals without previous urinary tract disorders* (Table IV) but again the observer differences in distinguishing leaky and disrupted erythrocytes from cellular debris of other origin may be large

The observer error in distinguishing leucocytes from non squamous epithelial cells may also be large but the error in counting is probably smaller when these cells are reported together (10) In about 95 % of *sterile* specimens from males and females without previous urinary tract disorders (group I) ⩽ 13 leucocytes + non squamous epithelial cells/mm³ were recorded which therefore may be regarded as a suitable upper limit for normal counts in routine examinations This limit was lower than for the whole series (Table IV) which also included bacterially contaminated specimens Again the random variability of haemocytometer counts must be considered (10) If for instance 13 cells/mm³ is an estimate of the true number about 7–22 cells may be found in 95 % of the occurrences on examining succeeding samples from the same specimen (5)

In females greater numb rs of white cells were generally found in bacterially contaminated than in sterile specimens (Table V) but of urines with ⩾ 21 white cells/mm³ specimens from all four males and two of eight females were sterile In the second specimen three males and three females had < 10 white cells/mm³ the respective numbers of white cells/mm³ in the two counts being 80 and 68 in one male 26 and 15 31 and 27 43 and 52 64 and 54 in four females From one female a second specimen was not obtained It is difficult to accept such results as normal Trichomonas vaginalis would probably by recorded as white cells in the cooled specimens Other unrecognized disorders might also be present but further studies were beyond the scope of this presentation

The urinary white cell count is of special interest in relation to the diagnosis of infections A high number of cells is th rule in acute infections in chronic pyelonephritis normal cell content has been reported by several authors (15 18 20 22) Table VIII shows that ⩽ 10 leucocytes + non squamous epithelial cells were recorded in about half of the specimens with ⩾ 10

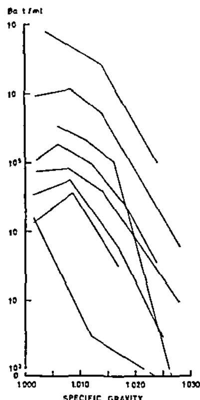


Fig 5 Rate of bacterial growth in urines of various specific gravities Eight urines diluted with distilled water Each dilution of the individual series was inoculated with the same amount of E coli suspension the amounts used in the different series being varied Incubation at 37 C for 270 min.

bacteria/ml Determination of the excretion of leucocytes is not a suitable test for bacteriuria but it may be a good screening test for urinary tract disease (8)

Although the main object of this investigation was to obtain an estimate of the cell content in non timed specimens of urine other points relating to bacteriuria and specific gravity of urine have emerged the lower bacterial counts (10^4 – < 10 bacteria/ml) were almost always made in specimens with specific gravities ⩾ 1 019 (Table VIII) A similar observation was made in a previous study (12) when the same amount of an E coli suspension was added to various dilutions of sterile urine in distilled water the concentration of bacteria in various dilution series being varied When quantitative culture was done after incubation at 37 C for 270 min optimal growth occurred at specific gravities of about 1 010 at the highest specific gravities the growth was apparently retarded or inhibited (Fig 5) The pH values ranged from 5 3 to 6 6 Similar observations have been made by Asscher et al (2) while Goldberg (13) reported that forcing fluid in preparation for bladder aspiration was associated with a fall in bacterial counts due to dilution The time factor and the amount of bacteria present beforehand may account for the difference In the evaluation of a low bacterial count in urine the specific gravity of the urine must be considered

REFERENCES

1 Aas K The cellular excretion in the urine of normal newborn infants Acta Pædiat (Uppsala) 50 361 *1961*

2 Asscher A W, Sussman M, Waters W E Harvard Davis R & Chick S Urine as a medium for bacterial growth Lancet 2 1037 1966

3 de Almeida S S Taunay A de E & de Alcantare L M Estudos sôbre as infecçoes urinárias nao especificas V Avaliaçao quantitativa da leucocituria em pacientes normais Rev Hosp Clin Fac Med S Paulo 16 181 1961

4 Brumfitt W Davies B I & Rosser I Urethral catheter as a cause of urinary tract infection in pregnancy and puerperium Lancet 2 1059 1961

5 Diem, K Documenta Geigy Scientific Tables 6th ed p 107 J R Geigy S A Basle 196?

6 Dubs B Die quantitative Ausscheidung geformter Harnbestandteile bei Nierengesunden und Nierenkranken Praxis 51 ?58 1962

7 Dukes C Some observations on pyuria Proc roy Soc Med 21 1179 19?7–?8

8 Fairly K F & Barraclough M Leucocyte-excretion rate as a screening test for bacteriuria Lancet 1 4 0 1967

9 Gadeholt H Erythrocytes and leucocytes in urine Variability of shape and size Univ Bergen Med avh 3 1968

10 — Counting of cells in urine The variability of haemocytometer counts Acta med scand 183 9 1968

11 — Persistence of blood cells in urine Acta med scand 183 49 1968

12 — Unpublished

13 Goldberg L M, Vosti K L & Rantz, L A Microflora of the urinary tract examined by voided and aspirated urine culture In Progress in pyelonephritis (ed E H Kass) p 545 F A Davis Co Philadelphia 1965

14 Houston I B Pus cell and bacterial counts in the diagnosis of urinary tract infections in childhood Arch Dis Childh 38 600 1963

15 Hutt M S R, Chalmers J A MacDonald J S & de Wardener H E Pyelonephritis Observations on the relation between various diagnostic procedures Lancet 1 351 1961

16 James, U Urinary infection in the newborn Lancet 2 1001 1959
17 Lam C N Bremner A D Maxwell J D Murphy A V & Low W J Pyuria and bacteriuria Arch Dis Child 4? ?75 1967
18 Leather H M Wills M R & Gault H M Bacterial pyrogen in diagnosis of pyelonephritis Brit med J 1 9 1963
19 Lincoln, K. & Winberg J Studies of urinary tract infections in infancy and childhood III Quantitative estimation of cellular excretion in unselected neonates Acta Pædiat (Uppsala) 53 447 1964
0 Little P J The incidence of urinary infection in 5000 pregnant women Lancet 2 925 1966
?1 Masters P L. Urinary changes in infections of the urinary tract in childhood Guy s Hosp Rep 10? 76 1953
22 Pears M A & Houghton B J Response of infected urinary tract to bacterial pyrogen Lancet ? 1167 1959
3 Reismann B Das normale Harnsediment des Menschen Inaug. Diss Koln 1964
.4 Rupp W Über die Leukocyten und Keimausscheidung im Urin gesunder Kinder bei Anwendung quantitativer Methoden Ärztl Wschr 14 13., 1959
5 Stansfeld J M & Webb J K G Observations on pyuria in children Arch Dis Childh 28 386 1953
.6 Sulheim O To be published
27 Teitel M Lambertson G H & Florman A L Filtration of urine for quantitation of cells and casts Amer J Dis Child 108 19 1964
.8 Winter K A & Knauth T Über das quantitative Harnsediment Zur Methodik und ihren Fehlern Med Wschr 11 154 1957

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CRYSTALLINE DIHYDROTACHYSTEROL (DYGRATYL®) IN THE TREATMENT OF HYPOPARATHYROIDISM

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Abstract Crystalline dihydrotachysterol has been used in the treatment of 26 patients with postoperative hypoparathyroidism The clinical experience has been favourable Dihydrotachysterol has advantages compared to calciferol, being faster in onset and fall-off of its effect, and can be given in an exact dosage Hypercalciuria seems to be common and urinary excretion of calcium should be determined regularly as well as serum calcium

Hypoparathyroidism has usually been treated with calciferol (vitamin D) or AT 10 Calciferol has the therapeutic disadvantage of an effect which is slow in onset and particularly in fall-off due to the long biological half life AT 10 consists of a mixture of irradiated sterols dihydrovitamin D_2II —the isomer of dihydrotachysterol—being the chief component (8) and is standardized in equivalents of dihydrotachysterol This standardization may vary by a factor of five (4)

It has been demonstrated in animals that crystalline dihydrotachysterol is more effective in raising serum calcium than calciferol (1) and more important has a more rapid effect and a considerably shorter biological half life These observations have been followed by clinical studies and crystalline dihydrotachysterol has been successfully used in the treatment of hypoparathyroidism (4 5) vitamin D deficiency (7 6 3) and vitamin D resistant rickets (5) The following report is concerned with our experience of crystalline dihydrotachysterol in the treatment of postoperative hypoparathyroidism

MATERIAL

The clinical material consists of 23 females and three males between -- and 75 years of age (Tables I and II) Three of the patients had had total parathyroidectomy because of generalized parathyroid hyperplasia Six had had total thyroidectomy and 15 had had bilateral subtotal thyroidectomy Two patients had had total hemithyroidectomy and subtotal thyroidectomy on the contralateral side with the lower part resected In all patients the diagnosis of postoperative hypoparathyroidism was established on the basis of low serum calcium, often in combination with increased serum inorganic phosphate (Fig. 1) In 19 patients urinary excretion of calcium was determined and in three of these a roentgenologic survey of the abdomen was performed with regard to renal calcifications (Table II)

Crystalline dihydrotachysterol (Dygratyl from Philips through AB Ferrosan) was administered in tablets containing 0 1 and 0.2 mg

Serum and urine calcium was determined with Eppendorf's flame photometer Serum phosphate was determined as inorganic phosphate soluble in acid (2)

RESULTS

In the beginning of the study small initial dosages (0 2–0 4 mg/day) were given which were gradually increased In the later part a high initial dosage (1 0–2 0 mg/day) was given to achieve normal serum calcium more rapidly When a normal serum calcium had been obtained the dosage was decreased until a satisfactory maintenance dosage was arrived at The serum calcium was normalized in all patients at a maintenance dose of crystalline dihydrotachysterol between 0 2 and 2 0 mg (Table II) With these dosages hypercalcemic episodes of short duration were observed in two patients (Figs 2 and 3)

Hypercalciuria which was defined as a urinary excretion exceeding 15 mEq/24 hours was observed in s ven of the 19 patients in whom determinations were performed In three of the seven patients with hypercalciuria roentgenologic

Table I *The clinical material with regard to type of surgery and clinical diagnosis*

Operation	Diagnosis	No of pats
Bilateral subtotal thyroidectomy	Non toxic goitre	3
	Toxic goitre	12
Total thyroidectomy one side + subtotal contralateral	Non toxic nodular goitre	4
Total thyroidectomy	Chronic thyroiditis	3
	Non toxic nodular goitre	3
Total parathyroidectomy	General parathyr hyperplasia	3
		26

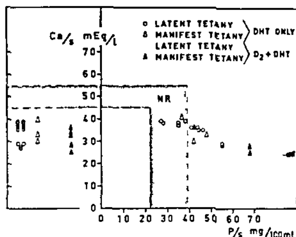


Fig 1 Serum calcium (*n* = 26) and serum calcium correlated to serum phosphate (*n* = 19) before treatment of postoperative hypoparathyroidism was started either with calciferol (closed symbols) or crystalline dihydrotachysterol (open symbols) Circles indicate patients with latent tetany and triangles patients with frank tetany Normal range for serum calcium 4 5-5.5 mEq/l for inorganic phosphate 2 2-3 9 mg/100 ml

surveys of the abdomen were made and did not reveal any renal calcifications

Nine patients had previously been treated with calciferol All nine declared that the treatment with crystalline dihydrotachysterol was easier to

Table II *Duration of treatment maximum and maintenance dosage urinary calcium excretion and serum calcium at time of determination of urinary calcium*

Duration of treatment (months)	Pat.	Sex	Age (y)	Maximum dosage (mg)	Maintenance dosage (mg)	Ca/u (mEq/24 h)	Ca/s (mEq/l)
3	A K	-	59	1 2	0 2	—	—
4	L F	-	34	1 6	1 2	—	—
5	G O	-	37	0 8	0 8	11 9	4 3
5	A A		55	1 0	0 8	15 3 12 2	5 4 5 0
6	A M		28	2 0	2 0	17 6[a]	4 7
8	K D		40	1 6	1 6	18 2 19 1 17 5[a]	4 6 4 6 4 6
8	E. A		56	0 8	0 8	2 3	5 2
9	S R	♀	44	0 4	0 4	5 0	4 9
10	T S	♂	22	1 3	0 8	14 5	4 9
11	S S		75	0 8	0 8	—	—
12	S Z		51	0 4	0 4	6 3	4 5
19	E. S		43	1 4	1 0	17 8 19 0	5 3 4 9
20	E. A		41	0 5	0 5	11 6	4 5
20	K M		30	0 8	0 8	10 1	4 8
21	A L.		65	0 8	0 8	18 0	4 7
27	A G		62	0 8	0 8	18 7	4 6
27	S A	♀	50	1 2	1 2	18 6	5 4
29	E N	♀	47	1 0	1 0	14 0	4 8
29	R O	-	50	1 0	1 0	21 6 19 3[a]	4 1 5 0
29	H P	-	30	0 6	0 6	11 0	4 5
29	W S	♀	35	0 5	0 5	6 3	4 8
29	E. N		74	0 4	0 4	—	—
30	S B		39	0 6	0 5	—	—
32	S D		62	0 6	0 5	—	—
34	I C.		33	0 4	0 4	—	—
34	A H		52	0 5	0 3	11 0	5 1

[a] Patients who have had a roentgenologic survey of the abdomen they showed no signs of renal calcification

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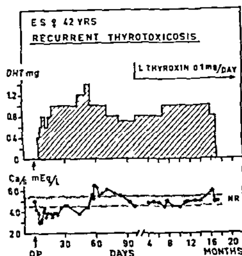


Fig 2 The course of treatment with crystalline dihydrotachysterol in a patient with postoperative hypoparathyroidism after second operation for thyrotoxicosis Note the rapid onset of the calcium mobilizing effect and the rapid fall in serum calcium with reduced dosage Normal range for serum calcium 4 5–5.5 mEq/l

support and gave them a greater sense of well being However only four had previously been in a reasonable steady state on calciferol The calciferol dosages used in these four patients were 0.5 1 2 1 4 and 1 5 mg/day A steady state was obtained with a dosage of dihydrotachysterol of 0 4 0 8 0 5 and 0 6 mg/day respectively

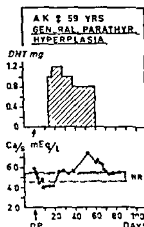


Fig 3 The initial course of treatment with crystalline dihydrotachysterol in a patient from whom four enlarged parathyroids had been removed Note the rapid fall in serum calcium when medication was stopped Normal range for serum calcium 4.5 5.5 mEq/l.

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cium in the two cas s presented (Figs 2 and 3) S

Dihydrotachysterol is administered in tablets c rather than a liquid solution This is apt to reduce the number of mistakes regarding dosage The patients generally found the tablets more convenient

Crystalline dihydrotachysterol has b en found to be more toxic in animal experiments than calciferol (1) This toxic effect was established on the basis of increase in serum creatinine and did not seem to correlate directly with the serum calcium level A number of the patients reported here had hypercalciuria (>15 mEq/24 h) in spite of a normal serum calcium and without biochemical signs of renal damage Consequently urinary excretion of calcium should be determined regularly and hypercalciuria should suggest a decrease in dosage of dihydrotachysterol

In this study only patients with hypoparathyroidism were included It seems however that dihydrotachysterol can replace calciferol in vitamin D deficiency (6 3) and vitamin D resistant rickets (5) Harrison et al (5) even observed that dihydrotachysterol is superior to calciferol in patients with steatorrh a This observation may indicate that the gastrointestinal resorption of dihydrotachysterol is less affected than that of calciferol in patients with steatorrhoea

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EVALUATION OF ATROMID S (CLOFIBRATE) *IN HYPERLIPIDEMIC STATES*

INTERIM REPORT

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Abstract Atromid S has been evaluated in an uncontrolled trial on a clinical material of hyperlipidemic states comprising 354 individuals, including an unusually high number of cases of essential hypercholesterolemic xanthomatosis Follow up periods varied between [illegible] and 4 1/2 years Side-effects reasons for withdrawal, persistency of lipid lowering effects rates of mortality and attacks of myocardial infarctions have been discussed A significant and persistent lipid lowering effect was seen in all varieties Although moderate this was also apparent in severe hypercholesterolemic xanthomatosis

Mortality and infarction rates were low—as was also the frequency of side-effects No serious side-effects were seen. The drug is considered to merit a considerable extension and prolongation of the study

Both Atromid (clofibrate with androsterone) and Atromid S (clofibrate) have in a great number of reports shown a reliable and fairly potent lowering effect on the serum triglycerides as well as a moderate effect on serum cholesterol Most of these reports include fairly small numbers of *patients belonging to different clinical subgroups* and have only covered a limited period Only the large British multi-centre trial reported by Green and Margetts (10) was of a size and duration sufficient to justify an attempt to evaluate rates of re infarction and deaths and to compare them with a number of previous materials Their material showed a promisingly low mortality and re infarction rate The conclusions of the authors were cautious owing to their lack of a control *material of their own*

Our experience covers a material of 354 patients followed for six months to four and a half years Although our study has neither the size nor the duration to give a definite idea of the long term prognosis we have considered an interim report of some interest for the following reasons

1 The material includes an unusually large number of cases of essential hypercholesterolemic xanthomatosis This group has been considered comparatively resistant to the action of Atromid S

2 The inclusion of 110 clinically asymptomatic cases with essential hypercholesterolemia and essential hyperglyceridemia should make an evaluation possible of the feasibility of using the drug on a primary prevention basis before clinical symptoms appear This would also give an idea of the numbers and reasons for drop-outs in a less urgently motivated group

3 Our own earlier long term study on the effect of strict diet covering observation times of 5–17 years in 112 hypercholesterolemic patients on a strict diet showed a strikingly low mortality as compared with an individually matched control series (13) Due to the surprisingly low mortality in absolute terms in the strict dieters there might even be a possibility that the addition of Atromid S to diet would change mortality in an undesirable direction It was thought that the present series would possibly give a preliminary idea about this and the results might then influence our future policy The reasons for running this study as an uncontrolled trial will be taken up in the discussion

REVIEW OF POSSIBLE MECHANISMS OF ACTION OF ATROMID S ON PLASMA LIPIDS

Although not pertinent to the main purpose of the present paper it may be appropriate to review shortly a

Table I *Distribution of material according to age sex lipid disturbance absence or presence and character of symptoms*

	Asymptomatic		Angina pectoris		Myocardial infarction		Two or more myocardial infarctions		Various clinical states		Sum
	≤50	≥51	≤50	≥51	≤50	≥51	≤50	≥51	≤50	≥51	
Males											
Hypercholesterolemia	3	6	3	11	6	13	—	2	1	2	47
Xanthomatosis	3	1	1	1?	2	5	2	—	—	2	8
Sum	6	7	4	23	8	18	2	2	1	4	75
Hyperglyceridemia	9	7	8	13	15	16	.	4	4	5	83
Borderline l pid values	3	—	—	3	3	5	—	1	1	2	18
Total	18	14	12	39	?6	39	4	7	6	11	176
Females											
Hypercholesterolemia	5	42	1	6	1	6	—	2	3	5	91
Xanthomatosis	8	4	3	10	2	4	—	1	—	4	36
Sum	13	46	4	36	3	10	—	3	3	9	1 7
Hyperglyceridemia	4	13	1	7	4	8	1	—	—	2	40
Borderline lipid values	1	1	—	3	—	—	—	—	4	2	11
Total	18	60	5	46	7	18	1	3	7	13	178

few facts that have emerged about the possible mechanisms of action of Atromid S

Ethyl α p-chlorophenoxy isobutyrate (CPIB Atromid S) has a plasma half life of about 1 hours The chloride substitution in the paraposition protects the compound from hydroxylation

Thorp (34) found CPIB in the rat to give a definite increase in the ratio of labelled thyroxine between liver and plasma He explained this by a displacement effect, as CPIB seems to be attached to the same binding site on albumin as thyroxine A number of the effects of CPIB seem to be explainable by an increased metabolically active concentration of thyroxine in the liver However this does not seem to explain all the known effects of the dru In contrast to thyroxine which stimulates the release of FFA from adipose tissu CPIB has been found to give a fairly strong and long lasting inhibition of the FFA release from the adipose tissue The serum FFA level shows a moderate but significant lowering

CPIB is known to have a more marked lowering effect on the serum triglyceride than on the serum cholesterol

In perfusion studies on the isolated rat liver using C palmitate Duncan et al (6) found about 50 decrease of the secretion rate of triglyceride from the liver under the action of CPIB In hyperglyceridemic human subjects Spritz (30 31) found a marked shortening of the plasma T½ for C labelled triglyceride glycerol in the very low density beta lipoproteins This was interpreted as an increase of the rate of removal of triglyceride from plasma affected by CPIB

Hood et al (1) have earlier reported an increase of the lipoprotein lipase activity found attached to the beta lipoprotein separated chromatographically in cold There was some inconclusive evidence that this might be due to suppression of inhibition of lipoprotein lipase

Persson and Hood (6) found an increase in the lipoprotein lipase activity of human adipose tissue only in three of fourteen subjects under the action of CPIB This has been studied against the background of the rather moderate variability shown on repeated biopsy samples from ?6 control subjects

Two groups Nestel et al (22) and Hollander and Chobanian (11) using in vivo experiments on hypercholesterolemic human subjects have found evidence which would suggest either inhibition of the biosynthesis of cholesterol or decrease in the utilization of freshly synthetized cholesterol for the formation of lipoproteins Isotope dilution calculations were interpreted as showing a decrease of the miscible pool of cholesterol in the study of Hollander and Chobanian (11)

OTHER EFFECTS OF ATROMID S

Other effects of Atromid S of possible therapeutic interest include a decrease of platelet adhesiveness (3 9 8) a decrease of plasma fibrinogen levels (5 3) and an increase of fibrinolytic activity (3) The relations of these various effects to each other and to the effect on the serum lipids seem not yet to be understood in any detail

CLINICAL MATERIAL

The material consists of 354 patients observed from six month to four and a half years

Table I gives information about the distribution of the clinical material according to sex, age type of lipid disturbance absence or presence and character of cardiovascular symptoms The heavy representation of cases in the decade 51 60 is seen There were few cases below 40 and very few above 70 for which reason the material was divided into age groups ≤50 and ≥51 4 per cent were ≤50 9 per cent ≥51 years of age In the group of essential hypercholesterolemia there were 64 cases of

Table II *Observation periods Numbers of withdrawals deaths and infarctions in the three major clinical groups in time relation to start of Atromid S*

Months	1–6	7–12	13–18	19–24	25–30	31–36	37–42	43–48	49–54	55–60	Total
Asymptomatic											
Taking Atromid S											
No of pat.	77	76	68	53	32	18	12	10	5	4	77
Deaths	—	—	—	—	—	—	—	—	—	—	—
Infarction	—	—	—	—	—	—	—	—	—	—	—
Stopped Atromid S											
No of pat	16	8	3	2	3	—	1	—	—	—	33
Deaths	—	1	—	—	—	—	—	—	—	—	1
Infarction	—	—	—	—	—	—	—	—	—	—	—
Angina pectoris											
Taking Atromid S											
No of pat	80	79	76	56	34	21	9	6	4	—	80
Deaths	2	—	—	—	2	—	—	—	—	—	4
Infarction	1	—	1	—	—	—	—	—	—	—	2
Stopped Atromid S											
No of pat	6	3	5	1	1	2	—	—	—	—	18
Deaths	—	1	—	—	—	—	—	—	—	—	1
Infarction	—	—	—	—	—	—	—	—	—	—	—
First myocardial infarction											
Taking Atromid S											
No of pat	62	60	54	47	29	18	13	4	2	—	62
Deaths	1	1	3	—	—	—	—	—	—	—	5
Re infarction	—	2	2	—	—	—	—	—	—	—	4
Stopped Atromid S											
No of pat.	8	7	4	3	1	—	—	—	—	—	23
Deaths	—	—	—	—	1	—	—	—	—	—	1
Re infarction	—	—	—	1	—	—	—	—	—	—	1

xanthomatosis of which 33 had widespread deposits in the tendons of all four extremities The grading used is given below

Xanthomatosis grade I Isolated xanthomata from the size of half a pea to a hazel nut in one or two dorsal tendons of a hand

Xanthomatosis grade II Hazel nut sized xanthomata or bigger in the dorsal tendons of both hands and in both Achilles tendons

Xanthomatosis grade III Huge deposits in the dorsal tendons of the hands and in both Achilles tendons (up to banana size) In addition large lumps in various locations i.e osseous in the peroneal tendons and in the dorsal tendons of the feet

The major emphasis has been placed on the three major sub-groups—asymptomatic angina pectoris and first myocardial infarction

The small clinical groups two or more myocardial

Table III *Observation periods mortality and hospitalized infarctions in Atromid series comparison with the British multi centre trial*

	No of pats	No of man months exposure	Death rate/100 man months	Myocardial infarction and re infarction rate 100 man months
British multi-centre trial				
Angina pectoris	13[illegible]	No information	0.16	No information
Myocardial infarction	312	6809	0.39	0.47
Present study				
Asymptomatic	110	2[illegible]71	0	0
Angina pectoris	10	66	0.18	0.09
Myocardial infarction Total	105	035	0.43	0.[illegible]
First myocardial infarction	90	1849	0.27	0
Two or more infarctions	15	36	1	0

Table IV *Deaths—Atromid S series*

Sex	Age	Initial state: Tendinous xanth grade	Asymptomatic	Angina pectoris	First myocardial infarction	Two or more myocardial infarctions	Time from myocardial infarction to start of Atromid S (months)	Months on/off Atromid S: On	Off	Lipid response	Type of death: Sudden	Infarction	Comments
ON ATROMID S													
Hypercholesterolemia													
♀	62	0	–	+	–	–	–	5		Good	+	–	Severe coronary atheroscler
♂	67	0	–	–	+	–	192	9		Sign	+	–	Severe coronary atheroscler
♂	50	II	–	–	–	3	½	21		Good	+	–	—
♀	44	III	–	–	+	–	½	6		Sign	+		Severe coronary atheroscler
♂	30	II	–	–	–	3	½	1		?	–	+	Severe coronary atheroscler Fresh thrombus
Hyperglyceridemia													
♀	70	0	–	+	–	–	–	27		Sign	–	–	Massive cerebral haemorrhage
♂	54	0	–	+	–	–	–	30		Good	+	–	Violent exertion
♂	32	0	–	+	–	–	–	5		Good	+	–	Violent exertion
♂	37	0	–	–	+	–	2	10		Good	+	–	Violent exertion Old infarction Fresh thrombus
o	36	0	–	–	+	–	3	14		Good	–	+	Recent infarction Fresh thrombus
♂	74	0	–	–	–	2	½	1		Poor	+	–	—
♂	46	0	–	–	–	2	36	4		Good	+	–	Recent infarction Fresh thrombus
o	39	I	–	–	–	2	9	25		Sign	–	+	Old and recent infarction
Border line lipid values													
♂	49	0	–	–	+	–	18	18		Good	+	–	—
OFF ATROMID S													
Hypercholesterolemia													
♀	53	0	+	–	–	–	–	7	3	Poor	–	–	Bronchial carcinoma
Hyperglyceridemia													
♂	36	III	–	–	+	–	Unknown	2	10	Poor	+	–	Severe coronary atheroscler
♀	66	I	–	–	+	–	39	4	24	Poor	+	–	Old and recent myocardial infarctions Fresh thrombus
Diabetic micro-angiopathy													
♂	6	0		–	–	–	–	2	10	?	+	–	—
♂	49	0	–	+	–	–	–	4	15	Good	–	+	—

infarctions claudication cerebro-vascular lesions renal artery stenosis and diabetes have been included in the overall data given on mortality reasons for withdrawal of the drug and the study of the white cell counts

The dosage of Atromid S was usually 0 25 g/10 kg body weight In a moderate number of cases a dosage as low as 0 1-0 15 g/10 kg body weight was used—in some cases with clear-cut effect on the serum lipids

More than two thirds of the patients were under the direct supervision of the authors The rest of the material was supervised by physicians with no direct active interest in lipid metabolism

RESULTS

Table II gives information on observation times and withdrawals deaths and infarctions As seen drop-out from treatment occurred usually rather early after introduction

Mortality and hospitalized infarctions

In Table III we have given the rates of deaths and hospitalized infarctions and compared with those

Table V *Hospitalized myocardial infarctions in Atromid S series*

Sex	Age	Initial state: Tendinous xanth grade	Asymptomatic	Angina pectoris	First myocardial infarction	Months from previous infarction	Months on Atromid S	Lipid response
Hypercholesterolemia								
♀	59	0	–	–	+	14	23	Good
♂	57	I	–	–	+	½	15	Good
♂	61	I	–	+	–	–	13	Good
♀	84	I	+	–	–	–	6	Fair
Hyperglyceridemia								
♂	4	0	–	–	+	½	7	Good
♂	51	0	–	–	+	1	12	Poor
♀	73	II	–	–	+	4?	17	Good

Withdrawn one month before the second infarction due to anorexia

reported from the British multi-centre trial where such comparison has been possible

The British multi centre series of myocardial infarctions included 18 6 of patients with multiple infarctions ours 14 3 The average age in our series was higher the lipid disturbance by our method of selection certainly more severe but the percentage of males somewhat lower No details on the severity of the lipid disturbance were available in the British report Still where the figures lend themselves to a comparison they seem very much alike Green and Margetts (10) found their figures for deaths and re infarction rates to be approximately half of those given for control materials collected from the literature on long term anticoagulant trials However in the absence of detailed documentation about the time elapsing from a previous myocardial infarction until the patients entered the various series it would be unwise to draw any firm conclusions It may be stated however that our figures are closely comparable to those of the British multi-centre trial and that they appear promising enough to encourage enlarging the series and continuing with those on treatment

Five patients died after withdrawal of Atromid S during a total exposure time of 1531 months making a death rate of 0 33/100 months As about two thirds of the months of exposure were represented by asymptomatic and angina pectoris patients this figure would appear somewhat higher than the average of those obtained during Atromid S The absolute figures for deaths in all subgroups concerned are naturally still definitely too small to allow any conclusions of real value

Table IV provides details on the patients who died while on Atromid S or after withdrawal of the drug One death was due to bronchial carcinoma. One female with extreme hyperglyceridemia and moderately severe hypertension died of massive cerebral haemorrhage The rest were either proven coronary or sudden deaths It seems noteworthy that death was sudden in 13 of these 17 cases In the ten autopsied cases coronary atherosclerosis was severe or extreme In three there were signs of recent infarction In five a fresh thrombus was found

As is seen from the lower part of the table there was no tendency to aggregation of deaths in a close time relation to the withdrawal of Atromid S

Details of the seven cases suffering from infarction which led to hospitalization have been given in Table V

As in the cases who died there seemed to be no tendency to aggregation during the first months on Atromid S The number of patients having had Atromid S for protracted periods for instance 2 years or more is still too limited to give any information on whether protracted treatment would tend to produce a lesser risk of infarction or death than in the early treatment period

Withdrawal of Atromid S

Table VI shows that Atromid S had been withdrawn in 25° of the cases The majority of these cases occurred in the first year of treatment as has been previously shown in Table II In more than half of the cases the drug was withdrawn on

Table VI *Reasons for withdrawal of Atromid S*

	On advice of physician	Respon sibility of patient	Total
Insufficient lipid reduction (includ 3 cases of chronic ethylism)	22	1	23
Anorexia gastric trouble	8	5	13
Too expensive	—	7	7
Chest pain worsened	1	3	4
Weight gain	1	—	1
Leucopenia	1	—	1
Re infarction	1	—	1
At admission to hospital	8	—	8
Lipids excellently con trolled	1	—	1
Reason not clear	6	6	12
Various causes	3	14	17
	57	36	88
Total number treated 354			
Withdrawals 85 (25)			

[a] These include impaired libido 1 psychic insuff headache 4 dizziness 3 going on journey 2 palpitation 1 itching 1 ischias 1 active reluctance 2 heard that people die of it 1

the advice of a physician The upper part of the table lists motives that appear to us comparatively well grounded The reasons s em to be the same as has been described in a number of publica tions The reasons given in the lower part of the table seem mostly to reflect lack of motivation on the part both of physician and patient Nearly all those patients were under the supervision of physicians who were only loosely associated with the team actively interested in lipid disorders In the group in which the drug had been withdrawn for reasons of insufficient lipid reduction there were four severely hyper triglyceridemic females who despite a rather substantial lowering of their triglyceride and cholesterol levels came within the range very commonly found in our material of myocardial infarction in early age groups We did not measure S_f 20–400 levels but tentatively considered the findings as a relative contraindica tion and withdrew the drug

The eight patients whose Atromid S treatment was dropped on admission to hospital usually for reasons unconnected with their lipid disturbance or cardiovascular disease represent a well known annoying feature

Short and long term lipid response

Oliver (24) has earlier produced good evidence that the lipid response was well maintained on Atromid S on a prolonged basis where no diet was prescribed As a great part of our material was on a rather strict low fat + polyunsaturated fatty acid diet when Atromid S was started two further problems might possibly be answered Would Atromid produce a further decrease of the serum lipids from the base line given on strict diet? During long term treatment with Atromid S

Table VII *Short and long term lipid response to Atromid S in 60 subjects on different diets*

				Atromid S 1–6 months		Atromid S 18 54 months	
	n	TG C	Before (mg)	(mg[a])	Reduction (%)	(mg)	Reduction ( )
Hypercholesterolemia							
Ordinary diet	6	TG	135	97	−30	99	27
		C	366	293	−20	310	−15
Strict diet	5	TG	117	83	− 6	85	− 4
		C	376	306	19	3 5	−14
whereof							
Xanth I	8	TG	104	85	18	88	15
		C	318	277	−13	283	11
Xanth II	6	TG	102	91	11	93	9
		C	398	366	8	366	− 8
Xanth III	5	TG	13	95	28	88	33
		C	494	339	−19	401	−19
Hyperglyceridemia							
Ordinary diet	11	TG	246	153	−38	172	30
		C	331	281	15	287	−13
Strict diet	18	TG	59	152	41	154	−40
		C	321	90	−10	299	7

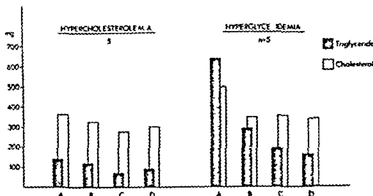


Fig 1 Serum lipid changes on strict diet as compared with a combination of strict diet and Atromid S after short and prolon ed treatment A ordinary diet B low fat + polyunsaturated diet C low fat + polyunsaturated diet + Atromid S 1-6 months D low fat + polyunsaturated diet + Atromid S 18-54 months

would the lipid response remain the same as initially as a tendency to slackening of the diet might occur—although the patients were encouraged to maintain their diet?

Sixty patients who had been on Atromid S for 25-54 months (average 37 months) and whose lipid data were adequate for assessment were used for this purpose The data have been given in Table VII and Fig 1 The reduction of lipid values in the late period (18-54 months) as compared with the early period (1-6 months) seems well maintained although a slight tendency to less reduction may be seen in most sub groups There seemed to be no greater tendency to a rebound in those on strict diet as compared with those who were treated with Atromid S and maintaining a normal diet

When the group of essential hyp rcholesterolemia cases was subdivided according to the presence or absence of tendinous xanthomata and according to the size and distribution of these lesions there emerged no definite trend showing any marked resistance to treatment in those with severe xanthomatosis

Thus Atromid S did produce a further lipid reducing effect from the base line of a strict diet This effect was essentially well maintained on a long term basis in the present group of subjects Surveying the individual data in this group of 60 subjects it seemed apparent that individuals obtaining a poor to moderate effect initially continued to do so also in the late period

Si le-effects

As has been seen previously from Table VI we have only encountered side effects of limited importance There were a number of cases in which the drug was withdrawn for different usually vague symptoms not described in the great number of publications on Atromid S that have ap p ared This has been interpreted as predominantly reflecting a lack of interest in the physicians responsible for instru ting and informing their patients about the purpose of the treatment

In a few patients there was an impression that chest pain worsened during treatment

As there have been a number of thorough studies previously dealing with the effect on liver function tests serum transaminases and coagulation parameters we early dropped systematic following of these measurements

Weight changes

There have been several reports of weight gain in materials of patients on ordinary diet We had sufficient data on 68 patients followed for more than two years who were on a strict diet

There was an average weight gain of about two kg This seemed moderate as compared with earlier reports and as possible mechanisms for weight gain have been rather thoroughly discussed earlier by Macmillan et al (18) we have refrained from further comments

White cell counts

Prout and Edwards (27) have described a case of agranulocytosis during Atromid S treatment in which however other drugs might also have been involved Engstrom (8) described moderate leucopenia in four out of eight subjects during a study of short duration

One of our patients exhibited a white cell count of 2000 Atromid S was withdrawn and has not been re introduced A systematic study

was made in 74 subjects of the material In a total of 412 observations on these 74 subjects the distribution was normal with a median value of 5500 w c Only 14 observations fell at any time below 3000 w c In two of these cases the low values prompted a temporary withdrawal of the drug which was later re introduced with per fectly normal counts for prolonged periods

In the 38 cases who have been followed syste matically for more than 2 years up to 4 1/ years there has been no sign of progressive lowering of the white cells in a single case Mean values for the group remained very stable between 5400–6200 w c per mm³ throughout the whole course There was not a single case of symptom produc ing granulocytopenia in the entire material

Other factors of possible importance for evaluation of the results

Hypertension under active antihypertensive treat ment was rare in this material and we have there fore refrained from analysis None in the asymptomatic and angina pectoris groups and rather few in the group of first myocardial infarction survivors were under anti-coagulant treatment when Atromid S was introduced In some of these the anti-coagulants were withdrawn after a pe riod There was no case of severe bleeding re corded in the material

DISCUSSION

An uncontrolled study such as the present one can evidently only give answers to a limited num ber of questions One may say that the drug was fairly effective in lowering serum lipids in most of the hyperlipidemic cases and that there were a number of cases with no recognizable effect If there was a reduction of lipid values this was protracted for up to 4 1/ years It would also seem to be clear that Atromid S provides a fur ther lipid lowering effect to that induced by diet Protracted treatment did not cause any signs of late toxicity or late troublesome side-effects When the drug was withdrawn it was done in approximately half of the cases for reasons which have been discussed in a number of earlier publications However in approximately half of the cases in which the drug was stopped the reason would seem to have no connection with the drug and sometimes not even with the underlying disease but seemed to be an effect of a lack of determination in the supervising physician Well instructed asymptomatic individuals with a biochemical derangement but without the urgent motivation of severe pain or fear of death have continued with the drug for many years

The important problem whether the mortality rate or rate of hospitalized infarctions was higher or lower than in previously existing materials can be only partly answered Definite conclusions can evidently not be reached without a randomized control material for several years to come Our group have had some reasons to refrain from the use of a randomized control material and a double blind placebo technique These reasons may be stated as follows Work on the effect of diet was started in comparatively young patients often with angina pectoris and a very strong family history of early myocardial infarction and death Here the question of randomization was from the outset an impossible one As observation pe riods increased in a number of these patients without new infarctions and angina pectoris in a number of patients disappeared or seemingly was ameliorated the start of randomization be came even more questionable We naturally re cognize the impossibility of registering improve ments of angina pectoris in an objective way to the satisfaction of others

We think that the position we have taken has been strengthened by the reports on long term trials of lipid reduction Eight of ten published long term studies on the effect of diet have re ported positive results (1 4 13 14 15 17 20 21) Several of these studies had obvious weak nesses including our own However the recently published studies of Leren were extremely pene trating and thorough on strictly randomized ma terial Morrison s and Hood et al s studies had certain drawbacks but represented the longest observation periods hitherto The two studies giving equivocal results were randomized but together with that of Koranyi (14) represented the short est observation times of all studies two and three years respectively Rose s (29) two-year study was on a small material which was further subdivided into three experimental groups It is probable that the diet method employed with addition of supplements of oil to the fat low cholesterol low diet led to the fact that only 16 12 and 15 subjects out of the 26 26 and 28 respectively in the initial

groups were stated to be left in the study at the end of the second year Although the method of selection methods of assessment and the statistical work up were of a high standard to us this study remains wholly inconclusive

The London Hospital Research Committee s study (16) with strictly randomized material on a low fat diet did not use polyunsaturated fat The diet group lost on an average ten the control group eight pounds of weight There was a reduction of serum cholesterol in both groups averaging about 40 mg% in the diet and 20 mg in the control group The curves showing the cumulative relapse rates showed for the diet group a flattening from the second to the fifth year (diminishing numbers of subjects) with only three relapses while the curve for the controls showed a steady rise Altogether it seems clear that some definite change of habits must also have occurred in the control group It would have been interesting to see a prolongation of this study for a further 4 5 or 6 years in order to reach definite conclusions Two of the three long term oestrogen studies have reported positive results (19 33) but the well executed five year study of Oliver and Boyd (25) was entirely negative One of the two prolonged heparin studies we have been able to find (7) also showed a reduction in death rates and re infarction The other one (2) showed a suggestive difference between the treated and the placebo treated group but this was not statistically significant

A survey of the present literature then has strengthened our opinion that the use of randomized material and a double blind placebo technique in a lethal disease often associated with severe pain and not rarely with severe anxiety may be questionable We fully realize that other approaches must necessarily give rise to considerable doubts as to the effect for several years to come We think however that something may be gained by using other methods of assessing results This might for instance include survival rates of consecutive cohorts of first myocardial infarction survivors studied closely before and after major changes in therapeutic approach

Further reports may in the meantime be expected from the British multi-centre trial The giant study on a randomized double blind placebo basis now well under way in Edinburgh may be hoped to give better information on the potential effect of lipid reduction with Atromid S in the asymptomatic males having serum lipids in the top section of the normal population There hardly seems to be any reason to try to copy studies of this type with limited resources We feel it well motivated to continue treating all definitely high risk patients of the type included in the present study

CONCLUSIONS

1 Atromid S has been evaluated in a material of 354 patients with essential hypercholesterolemia essential hyperglyceridemia and a small group with borderline lipid values Observation times varied between half a year and four and a half years

2 There were no deaths or infarctions in the clinically asymptomatic group of 110 patients

3 In the angina pectoris and myocardial infarction groups of 102 and 105 patients respectively the rates of deaths and myocardial infarctions were closely comparable to those obtained in the British multi-centre trial

4 In those who died while on Atromid S death was sudden in the majority of cases In the ten autopsies a fresh coronary thrombus was found in five

5 Atromid S produced a definite further lipid lowering effect from the base line obtained on a strict diet (low fat + polyunsaturated fat) The effect seemed well maintained over a period of up to four and a half years Although the group of severe essential hypercholesterolemic xanthomatosis cases appeared somewhat more resistant there was a definite further decrease in serum lipid as compared with that obtained on a strict diet also in this group

6 A little less than 25% stopped taking Atromid S the great majority during the first year of treatment The most common reasons given for stopping the drug appeared to be anorexia gastro intestinal troubles insufficient lipid reduction and economic reasons However in about half of the cases the drug was stopped for reasons that appeared less well founded and seemed mostly to reflect a lack of motivation in both physician and patient

7 The drug was withdrawn in a single case because of leucopenia (2000 w c /mm³) A systematic study of 74 patients of whom 38 were

followed for more than two up to four and a half years revealed no further cases and no progressive lowering of white cells in the group

8 Although no conclusions as yet may be drawn about the value of the drug in preventing deaths and infarctions the comparatively low rates recorded the safety and the fairly good lipid reducing effect appear to motivate extended use of the drug in the type of high risk individuals included in the present study It might also be considered to extend the use in groups carrying somewhat less risks

REFERENCES

1 Bierenbaum M L Green D P, Florin A Fleischman A I & Caldwell A B Circulation Suppl 2 3 1965
2 Bottiger L E Carlson L A Engstedt I & Oro L J Atheroscler Res 5 253 1965
3 Carson P McDonald L Pickard S Pilkington T Davies B & Love F J Atheroscler Res 3 619 1963
4 Christakis G, Rinzler S H Archer M & Maslansky E Publ Hlth Rep (Wash) 81 64 1966
5 Cotton, R C Wade E G & Spiller G W J Atheroscler Res 3 648 1963
6 Duncan C H Best M M & Despopoulos A Circulation Suppl 3 7 1964
7 Engelberg H Kahn R & Stenman M Circulation 13 489 1956
8 Engstrom J Svenska Lak Tidn 61 1445 1964
9 Gilbert J B & Mustard J F J Atheroscler Res 3 673 1963
10 Green K G & Margetts G Read in part at the 2nd International Symposium on Drugs Affecting Lipid Metabolism Milan 1965
11 Hollander W & Chobanian A V Circulation Suppl 2 18 1965
12 Hood B Bedding P & Carlander B J Atheroscler Res 3 509 1963
13 Hood B Sanne H Orndahl G Ahlstrom M & Welin G Acta med scand 178 161 1965
14 Koranyi A Ther hung 11 17 1963
15 Leren P Acta med scand Suppl 466 1966
16 London Hospitals Research Committee Lancet 2 501 1965
17 Lyon T P Yankley A Gofman J W & Strisower B Calif Med 84 3 5 1956
18 MacMillan D C Oliver M F Simpson J D & Tothill P Lancet 2 924 1965
19 Marmorston J Moore F J Hopkins C F Kauma O T & Weiner J Proc Soc Exp Biol Med 110 400 1962
20 Morrison L M J Amer med Ass 173 884 1960
21 Nelson A M North W Med (Seattle) 55 643 1956
22 Nestel P J Hirsch E Z & Couzens E A J Clin Invest 44 891 1965
23 Ogston C M Ogston D & McAndrew G M Curr ther Res 7 437 1965
24 Oliver M F Read in part at the 2nd International Symposium on Drugs Affecting Lipid Metabolism, Milan 1965
25 Oliver M F & Boyd G S Lancet 2 499 1961
26 Persson B & Hood B In preparation
27 Prout B J & Edwards E A Brit med J 2 543 1963
28 Robinson R W Read to Amer Coll Clin Pharmacol Chemother 3rd Ann Meetg Philadelphia April 1966 Abst in J New Drugs 6 126 1966
29 Rose H G Thomson W B & Williams R T Brit med J 1 1531 1965
30 *Spritz, N Circulation Suppl 2 201 1965*
31 — Diabetes 14 466 1965
32 Srivastava S C Smith M J & Dewar H A J Atheroscler Res 3 640 1963
33 Stamler J Pick R Katz L V Pick A Kaplan B M Berkson D M & Century D J Amer med Ass 183 63 1963
34 Thorp J M Revue de l athérosclerose et des artériopathies périphériques Arch Mal Cœur Suppl 1 2, 1966

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AUTONOMIC BLOCKING DRUGS ON CIRCULATORY ADAPTATIONS AT REST AND DURING EXERCISE IN MAN

Pronethalol and Poldine in Long term Treatment

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Abstract Oral doses of Pronethalol and Poldine were given to six subjects as blocking drugs for not less than one week A hemodynamic study was then repeated 1–3 hours after the last dose Heart rate intra arterial pressures right atrial pressure cardiac output pulmonary ventilation, oxygen consumption and respiratory exchange ratio were measured with the subject at rest in a recumbent and a sitting position, and during exercise sitting on a bicycle ergometer The mean values at rest were unchanged during blockade but the reactions of the variables when the patient was sitting up were changed for systolic and diastolic pressure oxygen consumption and arteriovenous oxygen difference The responses of heart rate and arteriovenous oxygen difference when the subjects changed from the recumbent to the seated position had lower variabilities during treatment. The left ventricular work and stroke work in the seated position had also lower variabilities during treatment than before During exercise the mean values for heart rate arterial pressures pulmonary ventilation and oxygen consumption were lower during blockade The variabilities of cardiac output stroke volume left ventricular work and stroke work and arteriovenous oxygen difference were also lower The changes in mean values from rest to exercise were reduced during treatment for heart rate arterial pressures left ventricular work pulmonary ventilation and oxygen consumption and the variability of reaction during treatment was higher for right atrial pressure but lower for left ventricular work. Similar studies which were made in each case with Pronethalol and Poldine respectively indicated that the present blockade was essentially additive Thus the most obvious effects were produced by Pronethalol but many of the orthostatic reactions were influenced by Poldine The exercise adaptation was well maintained during the present autonomic blockade

The autonomic nervous system plays a major part in the adaptation of the circulation to different activities Autonomic blocking drugs are used in the treatment of many disorders i e hypertension peptic ulcer disease

Pronethalol a potent β adrenergic receptor blocking agent has been tried in different rhythm disturbances (3 14 15) angina pectoris (2 14) as well as hypertension (10 13) Therapeutic use has been hampered by some adverse effects in laboratory animals (1 9) This drug was used however as the sympathetic blocking drug in the present study and Poldine a quaternary ammonium compound for peptic ulcer disease was chosen as the parasympathetic blocking drug

MATERIAL

Six male subjects aged from 21 to 54 years were selected for the study (Table I) Four of them attended the hospital for non bleeding peptic ulcer disease one for slight melena probably due to hemorrhagic gastritis as no wound was seen at the X ray investigation and one for evaluation of obesity (B B) One subject (K O) had moderate hypertension the others judging by their history physical examination or ECG showed no signs of circulatory disease

METHOD

The basal study was made in the morning in a post absorptive state A radiopaque catheter was introduced percutaneously into a cubital vein and advanced to the right atrium or a central vein under fluoroscopic guidance A polyethylene catheter was also inserted in the brachial artery using the same technique (Seldinger) About 10–20 mg of lidocaine without epinephrine were used as the local anaesthetic

After a rest period of half an hour in recumbency blood pressures were recorded, using variable inductance transducers (EMT 490 A Elema-Schonander AB) and a multichannel oscillograph ECG was recorded simultaneously Expired air collection was started via a low resistance system for about 5 min After about 2 min of air collection pressures were again recorded followed by

Table I *Presentation of some characteristics of the subjects*

Subject occupation	Diagnosis	Age	Height (cm)	Weight (kg)	Daily dose (g) over number of days Poldine
B B lift attendant	Normal subject (obesity)	26	182	130 8	0 040/8 + 0 10
H K excavator attendant	Peptic ulcer disease	35	186	86 0	0 016/5 + 0 024/5 + 0 032/3 + 0 040/15 + 0 010
K O cashier	Peptic ulcer disease Hypertension	52	171	71 6	0 016/3 + 0 0?4/1 + 0 032/1 + 0 040/2 + 0 010
G A cabdriver	Peptic ulcer disease	54	181	70 0	0 016/1 + 0 024/2 + 0 03?/18 + 0 008
I O plumber	Melena	33	186	100 5	0 016/4 + 0 024/2 + 0 032/2 + 0 040/5 + 0 010
B K workshop laborer	Peptic ulcer disease Hyperacidity	21	186	74 8	0 032/2 + 0 040/2 + 0 032/? + 0 040/1 + 0 010

a dye dilution determination of cardiac output Brom sulphalein was rapidly injected in the right atrium and fractional arterial sampling was simultaneously started as well as an ECG

After a rest period of half an hour these recordings were repeated with the subjects sitting in an armchair

After a similar rest period they started an exercise while sitting on an electrically braked bicycle ergometer Blood pressures and ECG were recorded every second minute After 5 min of work expired air collection was started and after 9–11 min of work the cardiac output procedure was applied

The work load of three subjects was 300 kg m/min that of one 400 kg m/min and that of two others 600 kg m/ n

hemodynamic study was repeated after treatment ?th Pronethalol and Poldine The mean daily dosage or Pronethalol was 0 73 g given for 10 days and the corresponding do e for Poldine was 32 mg for 14 days The last doses wer given together with a small amount of water about half an hour before the catheter was inserted These doses were 0 3 g in four and 0 . g in two of Pronethalol and 10 in five and 8 mg in one of Poldine

Five cm below the sternal notch was chosen as the zero pressure level in recumbency and the third inter co tal space as that in the sitting position Mean pressures were obtained by electrical integration

The expired air was analyzed for its oxygen and carbon dioxide contents using the Scholander apparatus and oxygen consumption respiratory exchange ratio and pulmonary ventilation were calculated

The arterial samples were centrifugated and the dye concentrations read from a B ckman B densitometer after alkalinization A dye concentration curve was plotted on semilogarithmic paper and the cardiac output calculated The hematocrit was determined after centrifugation at ?600 *g* for 15 min The stroke volume was calculated using the simultaneous ECG recording The total pe ripheral resistance was determined as the pressure dif ference between mean brachial arterial pressure and mean right atrial pressure in mm Hg over the cardiac output in l/min and expressed in arbitrary units The left ven tricular work and stroke work were calculated from the brachial arterial mean pressure times the cardiac output and the stroke volume respectively and expressed in both kg m/min and g m (grammeter) The pressures obtained immediately before the cardiac output procedure were used for all the calculations

Mean values and standard errors were determined ac cording to standard formulas Individual differences for corresponding recording were tested by *t* test *P* values are given for these differences Standard deviations were tested by *F* test

RESULTS

Mean values and standard deviations as well as *P* values for differences are given in Table II

Heart rate (HR beats/min)

No change in mean values was obtained during rest but the orthostatic reaction during blocking had a lower variability During exercise the heart rate was lower during treatment ($P < 0\ 05$) The increase from rest in the sitting position to ex ercise was smaller during treatment a difference which was even more marked ($P < 0\ 01$) The individual responses are shown in Fig I

The brachial arterial pressures (P_{BA_S} sy tolic P_{BA_D} diastolic and $\bar{P}_{BA}$ *mean pressure in mm Hg*)

The mean values at rest were *not* different dur *ing treatment* but when the changes from a re

and last dose Pronethalol	Interval after control study (days)	Symptoms during treatment
0 6/8 + 0 2	45	Dryness of the mouth Pal pitations
0 9/10 + 0 3	30	Dryness of the mouth Less peptic symptoms and less ap- prehended during treatment
0 6/2 + 0 9/8 + 0 3	12	Dryness of the mouth
0 6/15 ~ 0 2	23	Dryness of the mouth Diffi culties in starting micturition
0 3/1 + 0 6/2 + 0 9/5 + 0 3	12	Dryness of the mouth
0 3/1 + 0 6/1 + 0 9/2 + 0 6/2 + 0 9/1 + 0 3	7	Dryness of the mouth Diz ziness

cumbent to a sitting position were considered a small increase in diastolic pressure occurred in the sitting position during treatment as compared with a small decrease before treatment ($P < 0\ 02$) An indicative change ($0\ 10 > P > 0\ 05$) in the same dire tion for the systolic pressure i e a smaller positional change occurred but the change in the mean pressure and the pulse pres sure was not significant

During exercise all pressures during treatment were lower the decrease was most pronoun ed in the systolic pressure and then in the diastolic and pulse pressures When the changes from rest to exercise were considered increases in pressure were smaller during treatment these differences were even more pronounced ($P < 0\ 02$ for the smallest difference) The individual pressure re actions are given in Fig 2

The mean values of the right atrial pressures (RA mm Hg) were unchanged but the variability of atrial pressure change due to exercise was higher while under treatment

Flow-dependent variables

Cardiac output (Q l min) stroke volume (SV ml) peripheral resistance (SVR mm Hg l/min) left ventricular work (LVW kg m min) and stroke work (LVSW) (g m at rest were unchanged dur ing treatment.

Table II *Hemodynamic data on six subjects during rest in the recumbent and the sitting position and during exercise on bicycle ergometer before (B) and during (D) treatment with Poldine and Pronethalol*
Mean values standard deviations and P value ranges for differences For symbols see the text

	HR		P_{BA_S}		P_{BA_D}		P_{BA}		$P_{BA_S} - P_{BA_D}$		RA		Q		SV	
	B	D	B	D	B	D	B	D	B	D	B	D	B	D	B	D
Rest Recumbent	71 8	72.8	142 7	137 2	78 7	79 5	99 7	101 7	64 0	57 6	3 0	2 0	8 00	7 83	118	112
	21 4	14 8	27 3	16 4	8 2	5 7	17 4	12 2	19 4	11 4	1 2	1 7	1 7	1 4	39	35
Sitting	72 3	77 8	130 7	131 3	75 2	80 7	93 2	99 7	54 8	50 2	−2 5	−3 6	6 34	6 59	91	87
	13 7	12 6	19 6	11 0	8 6	4 7	10 9	9 3	12 9	6 4	2 7	2 3	1 62	1 19	32	22
Excercise	127 2	107 2	172 7	144 8	83 8	75 7	109	100 0	88 8	69 2	1 7	0 7	13 4	11 0	109	104
	23 7	15 1	18 1	12 7	3 5	7 6	7 0	8 0	18 7	12 2	4 1	3 6	4 6	1 7	46	18
	< 0 05		< 0 005		< 0 02		< 0 10		< 0 0.							

	SVR		LVW		LVSW		VE		VO_2		R		$(a-v)_O$		Hct	
Rest Recumbent	12 7	13 3	11 0	10 7	156	147	9 49	11 05	305	298	0 797	0 89	38 7	37 8	44 7	41 3
	3 5	4 1	3 5	1 7	40	44	.02	2 78	68	74	0 061	0 238	7 6	4 6	3 0	3 4
															< 0 025	
Sitting	15 9	16 4	8 0	8 8	115	116	11 95	11 22	333	292	0 840	0 835	54 3	45 0	45 3	4 7
	4 4	4 6	2 5	1 0	40	4	3 03	2 30	73	45	0 085	0 1 6	13 5	7 1	3 2	3 7
															< 0 05	
Excercice	8 8	9 3	20 1	14 9	163	142	42 62	37 54	1486	1318	0 903	0 905	119 5	1 0 5	48 0	45 0
	8	1 9	7 0	1 8	68	21	5 48	5 66	143	197	0 04	0 046	32 0	9 9	3 8	3 3
							< 0 10		< 0 10						< 0 10	

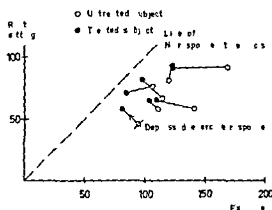


Fig 1 Heart rate reactions at rest and during exercise before and during blocking All treatment values are nearer the identity line where no reaction to exercise is obtained

The only effect during exercise on the mean values of these variables was an indicatively smaller increase in the left ventricular work from rest to exercise during treatment as well as a lower degree or variability also evident in the left ventricular stroke work

During treatment there was also less variation in the exercise recordings of cardiac output stroke volume left ventricular work and stroke work While the subjects were sitting at rest the variations in left ventricular work and stroke work were also smaller during treatment

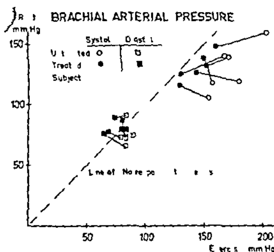


Fig 2 Arterial pressure reactions at rest and during exercise All the treatment values of systolic pressure are nearer the identity line where no reaction to exercise is obtained Some of the diastolic pressure reactions cross the line for treatment.

Respiratory dependent variables

The mean values for pulmonary ventilation (V_E l/min BTPS) oxygen consumption (VO ml/min STPD) respiratory exchange ratio (R) and arteriovenous oxygen difference (a-$\bar{v}$ O_2 ml/l) were unchanged during rest With regard to changing from a recumbent to a sitting position the small increase in oxygen consumption when the subjects were sitting up before treatment changed to a small decrease during treatment ($P<0\ 10$) The arteriovenous oxygen consumption was correspondingly changed i e the increase was less when the subjects sat up during treatment ($P<0\ 05$) On treatment during exercise variability in the arteriovenous oxygen difference was less than before

During exercise pulmonary ventilation and oxygen consumption were indicatively smaller during treatment The differences in the changes from rest to exercise reached the same level

Hemato rit (Hct) was somewhat smaller during treatment

The subjective working capacity was the same Dryness of the mouth was felt by all the subjects during treatment One (B B) complained of palpitations and another (G A) of micturation difficulties No ECG abnormalities were observed

DISCUSSION

The present method of studying the prolonged effect of circulatory active drugs seemed to be suitable judging by the good reproducibility obtained when the hemodynamic study was repeated (11)

Pronethalol tested alone by the same method pr du ed bradycardia and reduction in brachial systolic and diastolic pressure during exercise comparable to that obtained in the present study (13) In the present study however the reduct on in the brachial arterial mean pressure was only indicative during exercise which applied also to the decrease in oxygen consumption The same change in positional response regarding brachial diastolic pressu e when the subject was sitting up was evident when Pronethalol was given alone Variations in these responses differed being higher for heart rate and lower for systemic vascular res stance The variabilities in left ventricular work and stroke work were also lower however after Pronethalol alone No changes in positional re

sponses in respe t of systolic pressure oxygen consumption arteriovenous oxygen difference were observed when Pronethalol was given alone

When considering the changes from rest to exercise Pronethalol given alone also produced a smaller increase in heart rate systolic and diastolic pressure oxygen consumption and left ventricular work ($P < 0\ 10$) but had no significant effect on the mean pressure

According to another report Poldine given alone p oduced increased heart rate and brachial arterial dia tolic pressure ($P < 0\ 10$) in the sitting position (12) These changes were not observed in the present study In recumbency at rest Poldine decreased the oxygen consumption and arteriovenous oxygen difference These changes were not detectable in the present study There was also an indicative rise in cardiac output. Poldine alone also exaggerated the positional changes in heart rate br chial arterial mean pressure blood flow and right atrial pressure These changes were also abolished in the present study when Pronethalol was added Variability during Poldine treatment was less at rest in recumbency only for left ventricular work whereas in a sitting po ition it was also less for stroke volume During exercise there was also less variation in these variables and in systolic and mean pressure cardiac output and stroke work

The Poldine and Pronethalol doses given in this study were comparable with the doses administered when each drug was tested alone (12 13)

Thus when the subje t was sitting up changes in the adaptation of the circulation during this blo kade of the autonomic nervous system could be explained by the β adrenergic blo kade of Pronethalol Some of these effects were however counteracted by Poldine Most of the changes in the adaptation to work could be explained in the same way but the decrease in variability might have been due to Poldine

When both drugs were administered arterial pressure reaction during exercise was at least as small as when Pronethalol or Poldine was given alone This may indicate that compensatory reflex me hanisms from the other autonomous divisions are operative when one part is blocked However the α re eptor part responsible for the resistance vessel reactions was not shown to b blocked by Pronethalol which makes the d crease in arterial pressure reaction difficult to explain The a tive dilatation of resistance vessels m diated by the β adrenergic system mu t also be blocked this makes the pressure rea tion still more difficult to explain Some intrinsic β sympathetic activity of the drug may account for this

A more complete blocking by drugs of the autonomic nervous system in man was investigated by Kahler et al (8) They studied the adaptation to work in recumben y during high peroral do es of Guanethidine and a high intravenous dose of Atropine They obtained a smaller increase in heart rate an inversion of the arterial pressure reaction and a smaller cardiac output increase during exercise After administration of the block ing drugs their values during exercise for stroke volume and ventricular stroke work were lower than before Atropine when given alone did not qualitatively change the response to exercise consequently they concluded that the sympathetic division plays a major role in this adaptation However Atropine when given intravenously has initially the effect of increasing cardiac output heart rate and blood pressure (16) but later (15 min or more) the only effect observed is increased heart rate (4) This may depend on different delays after injection before blocking at th various circulatory reflex stations Unlike Atropine Poldine is not regarded as penetrating the blood brain barrier Consequently with Poldine only peripheral blo king of the parasympathetic influence can be accomplished

After high i v doses of Atropine and Propranolol the cardiac reactions to vagal and sympathetic stimuli were almost entirely eliminated and an increase of heart rate which was most marked in young and healthy subje ts wa observed during rest (6 7) The arterial pressure rose but cardia output was not affe ted (7) The term intrinsic heart rate was used to denote this autonomic "isolation of the heart

No similar effects on heart rate or blood pressure were seen in this long term study The blocking doses were also not comparable

The smaller variability of the flow reactions during exercise observed in the present material was probably an effect of autonomic blo kade This may indicate that the autonomic nervous system is not of ma or importance for adaptation to work It may only reinforce or weaken the response in individual subjects and thus cause greater variability in the responses during exercise

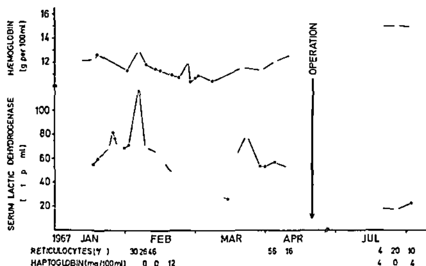


Fig 1 Hematological changes in a 48 year old woman with aortic valve disease The hemolysis subsided after operation

At combined mediastinal and left ventricular puncture the pressure in the aorta was 1?4/62 mm Hg and in the left ventricle 250/42 mm Hg without any gradient across the mitral valve

Thoracic aortography confirmed the diagnosis of severe valvular aortic stenosis with moderate aortic incompetence and heavily calcified aortic valve

Course during admission

On admission she had a hemoglobin of 12 2 g/100 ml (Fig 1) a sedimentation rate of 2? mm/hour a white cell count of 11 200/μl She had a fever rising to 39 5 C in the evening subsiding spontaneously within four days A few coli bacteria were cultured from the urine

One day after the right heart catheterization—which took place on the 8th day after admission—the serum lactic dehydrogenase which was then determined for the first time was 55 U/ml and continued to be elevated ranging between 110 U/ml and 47 U/ml throughout hospitalization which lasted three months (Fig 1)

The elevation was due to isoenzymes no 1 and 2 but as it was constant and neither the patient s clinical condition nor the ECG changed suspicion of myocardial damage as cause of the elevation was soon abandoned

Four weeks after admission she again had fever up to 39 4 C lasting for 16 days and then subsiding spontaneously No other focal signs of infection were observed except for moderate pyuria The hemoglobin fell slightly to 11 4 g/100 ml There were no signs of bleeding The laboratory tests were as follows

The red-cell count was 3 500 000/μl the mean corpuscular volume was 91 fl the mean corpuscular hemoglobin concentration 35 g/100 ml the hematocrit 3? the reticulocyte count 30–26–46‰ (Fig 1) the serum iron 46 μg/100 ml the serum iron binding capacity 269 μg/100 ml the serum B 480 μg/100 ml the folic acid normal the serum haptoglobin values were 0–0–12 mg/100 ml (Fig 1) the bleeding time the coagulation time the prothrombin content and the thrombocyte count were all within normal limits

The serum albumin was slightly decreased (3 28 g/100 ml) and the alkaline phosphatase elevated (66–61–45 U/100 ml the upper normal limit being 38 U/100 ml) the serum bilirubin was normal The serum electrolytes and serum creatinine were within normal limits The protein bound iodine was 6 0 μg/100 ml and the serum cholesterol ranged between 163 and 279 mg/100 ml The erythrocyte glucose-6-phosphate dehydrogenase was 156 pico-units per erythrocytes (normal value 88–151 pico-units)

Histologic examination of a sternal marrow specimen revealed hyperplastic normoblastic erythropoiesis

The conclusion was that the patient had hemolysis of unknown cause She received no drugs that could cause hemolysis The erythrocyte osmotic fragility was normal The direct antiglobulin (Coombs) test Ham s and Crosby s tests and direct Donath-Landsteiner test were all negative and tests for cold and heat agglutinins were negative

Subacute bacterial endocarditis was strongly suspected as the cause of hemolysis in this patient However negative results were obtained from nine bloodcultures (seven of which were from arterial blood) and from culture of the bone marrow and this condition was then ruled out

It was then supposed that chronic hemolysis might be explained by mechanical damage of the erythrocytes when passing the stenotic aortic valve On the peripheral blood smear a number of bizarre erythrocytes and fragments of erythrocytes were seen

Operation

The cardiac condition of the patient remained unchanged in spite of diuretic treatment in addition to the treatment with digitalis and she was referred for operation

At operation during extracorporeal circulation the ascending aorta was widely dilated measuring up to 7 cm in diameter The outer diameter of the aortic root was 34 mm The aortic valve was heavily calcified and there was agglutination between the left and the right coronary cusp so that the valve appeared as a bicu pid valve with a split opening of 18 x 3 mm A forcible jet was felt in the aortic arch before perfusion was started After resection of the aortic valve the cusps were found to be about

Table I *One hundred and two patients with rheumatic and congenital heart disease investigated for hemolytic anemia*

	No of pats	Hemolysis
Congenital[a]	3[illegible]	0
Mitral valvular disease	28	0
Aortic stenosis	13	3
Aortic insufficiency	4	0
Aortic stenosis and aortic insufficiency	10	2
Aortic and mitral valvular disease	15	2
Total	102	7

[a] Except for aortic valvular disease

1 cm in thickness with uneven surface A homograft was inserted

The patient s clinical condition improved considerably

Follow up

Three months after the operation renewed hematological studies were undertaken the following values being obtained at an interval of one week (Fig 1) hemoglobin 15 0–15 1–15 0 g/100 ml reticulocytes 4–[illegible]0–10‰ lactic acid dehydrogenase 18–17–22 U/ml haptoglobin 4–0–4 mg/100 ml serum iron 93–91–104 μg/100 ml transferrin 241–[illegible]6 – 60 μg 100 ml

MATERIAL AND METHODS

One hundred and two patients with rheumatic and congenital heart disease admitted to this department during a four month period were investigated The distribution on diagnosis is seen in Table I

Seventeen of the [illegible]8 patients with isolated mitral valve disease had previously had mitral valvulotomy The 3[illegible] patients with congenital heart disease consisted of 1 with atrial septal defect eight with ventricular septal defect five with pulmonary stenosis four with Fallot s tetralogy and three with coarctation of the aorta

The hemoglobin level reticulocyte count serum lactic dehydrogenase serum haptoglobin and plasma hemoglobin were determined at least twice with one day in between The normal values in this laboratory are hemoglobin males 13 2–17 6 g/100 ml females 11 7–15 7 g/100 ml reticulocyte count < 12‰ serum haptoglobin 35–170 mg/100 ml serum lactic dehydrogenase 7–[illegible]3 U/ml plasma hemoglobin not established

A diagnosis of hemolysis was established when a stable or decreasing hemoglobin level was found together with reticulocytosis in the absence of bleeding

The patients who thus showed signs of hemolysis were followed for at least two weeks with frequent determinations of the above mentioned values In patients with a mean reticulocyte count of ≥ 20‰ the ^{51}Cr red cell survival half time (Cr T½) was determined (normal value [illegible]2–31 days) Furthermore in the hemolytic patients a number of additional laboratory analyses were made serum bilirubin serum iron and serum iron binding capacity serum B_{12} serum folic acid direct antiglobulin (Coombs) test direct Donath–Landsteiner s test Ham s and Crosby s tests serum hemolysins at 37 C osmotic fragility of the erythrocytes leucocyte count and thrombocyte count The peripheral blood was investigated for schistocytes and the bone marrow was examined

RESULTS

Hemolysis was found in seven patients (Tables I and II) Five of the patients had isolated aortic valve disease while the remaining two had aortic valve disease in combination with slight mitral valve disease

In none of the patients with isolated mitral valve disease or congenital heart disease apart from aortic valve disease could hemolysis be demonstrated

None of the seven patients with hemolysis had iron deficiency folic acid or vitamin B_{12} deficiency The leucocyte counts and distribution and the thrombocyte counts were within normal limits The direct antiglobulin (Coombs) test Donath–Landsteiner s test Ham s test and Crosby s test

Table II *Studies of seven patients with mechanical hemolysis*

Pat	Diagnosis	Sex	Age (y)	Hemoglobin (mean) (g 100 ml)	Reticulocytes (mean) (‰)	Serum haptoglobin (mg/100 ml)	Serum LDH (units ml)	^{51}Cr T½
H I	AS AI	♀	58	10 4	28	4–8	21	0
J O	AI MI	♀	34	10 9	6	1 –71–90 104–8	20	19
A A H	AS	♂	52	13 9	4	0–24–90	31	19
H J	AS AI	♂	56	11 6	0	0–0–4–4	19	--
N H	AS		45	[illegible]	17	0–0–0–8 28	0	
P S	AS	♂	53	15 3	17	0–4–16–12 12 8	4	
A H	AS AI MS		57	[illegible]	[illegible]	0–0–11–15	17	

MI = mitral insufficiency MS mitral stenosis LDH lactic acid dehydrogenase AI = aortic insufficiency AS aortic stenosis

were all negative and no serum hemolysins were found The osmotic fragility of the erythrocytes was normal in all cases

The peripheral blood of all the seven patients with hemolysis contained a small number of schistocytes and erythrocyte fragments and the bone marrow showed hyperplastic normoblastic erythropoiesis

Table II shows that four of the seven patients had anemia while the hemolytic disease was compensated for in the remaining three The ^{51}Cr T½ was slightly decreased in three patients and was in the lower part of the normal range in one (H J) The serum bilirubin was elevated in one patient (A A H) on admission (1 8 mg/100 ml) but subsequently was normal (0 4 mg/100 ml)

Patient J O was admitted with a reticulocyte count of 52‰ and a serum haptoglobin value of 12 mg/100 ml During her stay in the department where most of the time she was bedridden the reticulocyte count dropped to 12‰ while the serum haptoglobin value rose to 104 mg/100 ml the hemoglobin level was stable at 10 9 g/100 ml At a later admission from her home where she had been active in housekeeping the reticulocyte count had again increased to 48‰ while the serum haptoglobin value was 8 mg/100 ml

The plasma hemoglobin level in all 102 patients was between 0 and 8 mg/100 ml there was no difference between the values for patients with and without hemolysis

DISCUSSION

The possibility of traumatic hemolysis in patients with unoperated valvular heart disease was first suggested by Dameshek in 1964 (5) He discussed a case of hemolytic anemia in a 63 year old woman with calcified aortic and mitral valve disease

Thereafter the question of traumatic hemolysis in these patients was investigated with determination of ^{51}Cr erythrocyte survival time by Brodeur et al (2 3) Yacoub et al (16) and Gehrmann et al (7)

Brodeur et al (2) found shortened ^{51}Cr T½ in 18 of 21 investigated patients with unoperated aortic valve disease He states that although none of the patients was anemic the findings for one patient were compatible with compensated hemolytic anemia This patient however, had previously undergone aortic commissurotomy

Yacoub et al (16) investigated 11 patients with aortic valve disease and found normal values of the ^{51}Cr T½

Gehrmann et al (7) observed shortened ^{51}Cr T½ in 18 of 37 patients with different valvular heart diseases Ten of the patients had isolated aortic valve disease two had combined aortic and mitral valve disease and the remaining six had isolated mitral valve disease None of the 18 patients was anemic and the hemoglobin level in the six with isolated mitral valve disease ranged between 14 4 and 17 0 g/100 ml

Brodeur et al (3) observed only a slightly shortened ^{51}Cr T½ in four of 14 patients with unoperated isolated mitral valve disease none of these patients was anemic

Determination of serum haptoglobin has been used by Veneziale and associates (14) and Cullhed (4) to discover low grade hemolysis

Veneziale and associates (14) found decreased value of serum haptoglobin in six of 24 patients with unoperated valvular heart disease The hemoglobin value was only mentioned in respect of three of the six patients it was normal The reticulocyte count was not determined preoperatively Cullhed (4) found low serum haptoglobin values in two of 26 unoperated patients with aortic valve disease Neither of the patients had anemia the reticulocyte count is not stated

In a study of serum lactic dehydrogenase level in patients before and after cardiac catheterization Jorgensen et al (9) found elevated values in ten of 43 patients with different unoperated congenital and rheumatic heart diseases They suggest that the elevated values could be attributed to a low grade compensated chronic hemolysis

In several other reports traumatic hemolysis has been suggested (11 15 17)

Miller et al (11) found traumatic hemolysis in a 27 year old man with rheumatic aortic and mitral valve disease The degree of hemolysis as determined with ^{51}Cr T½ increased with increasing muscular activity of the patient

Westring (15) reports the condition in a young woman with aortic insufficiency In a further prospective investigation of 12 patients with aortic valve disease traumatic hemolysis was present in one anemic patient in two other patients the ^{51}Cr

T3 was slightly shortened but there was no other evidence of hemolysis.

So far four cases of hemolytic anemia have been described in patients with unoperated valvular heart disease (5, 11, 15). All four patients had aortic valve disease but two had in addition mitral valve disease.

The patient with compensated hemolysis reported by Brodeur et al. (2) had previously been operated on for aortic stenosis with commissurotomy. In a case described by Ziperovich and Paley (17) hemolytic anemia developed after unsuccessful mitral valvuloplasty; at a second operation the anterior leaf of the mitral valve was found to be destroyed and the anemia disappeared after a Starr-Edwards valve had been inserted. This anemia corresponds to the severe hemolytic anemia developing in patients with postoperative prosthetic valve defects (6-10).

In our material hemolytic anemia was found in five patients with valvular heart disease. Furthermore three patients had compensated hemolysis. All eight patients had aortic valve disease as the predominating abnormality while two had also slight mitral valvular disease.

Our findings and the previously reported cases indicate that unoperated mitral valve disease is hardly likely to provoke traumatic hemolysis.

One of our patients (J. O.) demonstrated increasing hemolysis with increasing physical activity, which occurred also in the case described by Miller et al. (11).

REFERENCES

1. Brain, M. C., Dacie, J. V. & Hourihane, D. O'B.: Microangiopathic haemolytic anaemia. The possible role of vascular lesions in pathogenesis. Brit. J. Haemat. 8: 358, 1962.
2. Brodeur, M. T. H., Sutherland, D. W., Koler, R. D., Starr, A., Kimsey, J. A. & Griswold, H. E.: Red blood cell survival in patients with aortic valvular disease and ball valve prostheses. Circulation 32: 570, 1965.
3. Brodeur, M. T. H., Koler, R. D., Starr, A. & Griswold, H. E.: Red cell survival in patients with mitral valvular disease and mitral valve replacement. Circulation, Suppl. 2: 8, 1965.
4. Cullhed, I.: Serum haptoglobin in cases with Starr-Edwards ball valve prosthesis. Acta med. scand. 181: 3, 1967.
5. Dameshek, W.: Case records of the Massachusetts General Hospital. Case ...-1964. New Engl. J. Med. 271: 898, 1964.
6. DeCesare, W., Rath, C. & Hufnagel, C.: Hemolytic anemia of mechanical origin with aortic-valve prosthesis. New Engl. J. Med. 272: 1045, 1965.
7. Gehrmann, G., Bleifeld, W. & Kaufen, D.: Herzklappenfehler und Hämolyse. Klin. Wschr. 44: 1229, 1966.
8. Guigan, D. R. & Baumgart, H. L.: March hemoglobinuria. Medicine 20: 341, 1941.
9. Jorgensen, C. R., Zimmerman, T. S. & Wang, Y.: Serum lactate dehydrogenase elevation in ambulatory cardiac patients. Circulation 35: 79, 1967.
10. Marsh, G. W.: Intravascular haemolytic anaemia after aortic-valve replacement. Lancet 2: 986, 1964.
11. Miller, D. S., Mengel, C. E., Kremer, W. B., Gutterman, J. & Senningen, R.: Intravascular hemolysis in a patient with valvular heart disease. Ann. intern. Med. 65: 210, 1966.
12. Rose, J. C., Hufnagel, C. A., Fries, E. D., Harvey, W. P. & Partenope, E. A.: The hemodynamic alterations produced by a plastic valvular prosthesis for severe aortic insufficiency in man. J. clin. Invest. 33: 891, 1954.
13. Sohlman, F., Jr, Sarnoff, S. J., Case, R. B. & Ness, A. T.: Hemolytic syndrome following the insertion of a lucite ball valve prosthesis into the cardiovascular system. Circulation 13: 86, 1956.
14. Venezale, C. M., McGuckin, W. F., Hermans, P. E. & Mankin, H. T.: Hypohaptoglobinemia and valvular heart disease. Association with hemolysis after insertion of valvular prostheses and in cases in which operation had not been performed. Mayo Clin. Proc. 41: 657, 1966.
15. Wesling, D. W.: Aortic valve disease and hemolytic anemia. Ann. intern. Med. 65: 203, 1966.
16. Yacoub, M. H., Rowers, K. & Taylor, P. C.: Red cell survival in patients with aortic valve disease. Thorax 20: 367, 1965.
17. Ziperovich, S. & Paley, H. W.: Severe mechanical hemolytic anemia due to valvular heart disease without prosthesis. Ann. intern. Med. 65: 342, 1966.

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LACTIC ACID ACCUMULATION IN CONNECTION WITH FRUCTOSE INFUSION

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Abstract Infusion of fructose in healthy experimental subjects and in patients with diabetes mellitus is found to cause an increase in the blood concentration of lactate that is in relation to the rate of infusion. At an infusion rate of 1 g/kg BW/h or above the lactate concentration rises by 5 mEq/l or more In healthy subjects infusion is generally associated with a relatively inappreciable rise in arterial glucose concentration In diabetic patients on the other hand, the rise is pronounced Clinically significant acidosis is found to be exacerbated by rapid fructose infusion. It is therefore emphasized that this form of treatment is contraindicated in acidotic states Administration of fructose has no beneficial effect on the metabolism in insulin requiring diabetic patients whose insulin has been temporarily discontinued On the contrary it exacerbates their acidosis and produces a considerable rise in blood sugar

During the past few years fructose has started to be used to a great extent instead of glucose for parenteral nutrition with carbohydrates In recent survey articles fructose is stated to have many advantages over glucose for this purpose (e g 19)

Fructose is more rapidly metabolized resulting in a lower total sugar concentration in the blood than when the corresponding amount of glucose is given (2 17 20) It has been reported that postoperative patients and uraemic patients with glucose intolerance can utilize fructose in a normal way (6 13) Since fructose is not dependent on insulin for intracellular penetration it has been considered of value in cases of diabetic acidosis or after total pancreatectomy (5 16 17) Moreover fructose is stated to have a less irritant effect on peripheral veins than glucose which implies that fructose solutions for intravenous use can be given in higher concentration (19)

An account is given in this paper of a study of the way in which infused fructose affects the lactic acid concentration in blood both in healthy experimental subjects and in patients with diabetes mellitus We had two aims One was to ascertain whether significant lactic acidosis could be induced by fructose infusion The other was to determine whether fructose had any beneficial effect on the metabolism in diabetic patients whose insulin had been temporarily discontinued

MATERIAL AND METHODS

Seven healthy experimental subjects and three patients with juvenile diabetes mellitus were investigated in the morning after an overnight fast An indwelling catheter was inserted in the brachial artery for blood sampling The healthy subjects were given a continuous infusion of 20 fructose The rate of infusion was 0 4 g kg BW/h in one case (M A) and 1 0-1 3 g/kg BW h in four (M V A A A F and E J) The infusion proceeded for two hours or longer Two subjects (M H and M R) were given a more rapid infusion (2 2 and 3 5 g/kg BW/h respectively) but in these cases administration of fructose was discontinued after an hour in view of subjective complaints in the form of nausea and precordial pain

The metabolism of the splanchnic region was studied by means of hepatic vein catheterization in four subjects (M H M R A F and E F) The results of these studies have been reported elsewhere (2)

Two of the diabetic patients had been without insulin for 7? h and the third patient (M C) for 48 h before the investigation

M C and A L were given a fructose infusion of 1 2 g/kg BW/h during 3 ½ h After an interval of 90 min A L was given 0 IU of rapidly acting insulin intravenously followed by a fructose infusion at the same rate for 3 ½ h

A fructose infusion (0 6 g kg BW h) was started in case C K After 30 min hyperventilation appeared and she became somnolent Rapidly acting insulin (25 I U) was given intravenously and after a dose of 20 g of fructose in the course of 5 min the infusion was stopped and bicarbonate solution administered (100 ml of 1 3 solution in the course of 50 min) A fresh attempt at infusing fructose (1 g kg BW h) also had to be dis

Table I *Changes in blood glucose fructose and lactate in connection with fructose infusion*

Values obtained after 2 h of continuous infusion in all cases except M H and M R in which the infusion was stopped after 1 h

Subject	Sex	Age (y)	Weight (kg)	Infusion rate of fructose (g/kg BW/min)	Δ Arterial glucose concentration (mg/100 ml)	Δ Arterial fructose concentration (mg/100 ml	Δ Lactic acid concentration (mEq/l)
Normals							
M A	♂	22	84	0 5		44	0 9
M V	♀	18	53	1 1	14	93	5 0
A A	♂	38	72	1 0	−13	107	5 0
A F	♀	24	59	1 0	33	64	5 5
E J	♀	22	60	1 3	61	104	6 7
M H	♂	2[illegible]	70	2 2	38	178	6 5
M R.	♀	22	51	3 5	35	383	8 0
Diabetics							
A L	♂	17	57	1 2	105	112	5 4
M C	♀	16	50	1 2	265	132	5 3
G K	♀	13	41	0 9	—[a]	75	3 7

[a] Insulin administered

continued because of increasing symptoms of acidosis During the subsequent part of the experiment insulin was administered repeatedly i v Fructose infusion was then restarted (0 6 g/kg BW/h) and continued for 3¹/ h The blood pH and standard bicarbonate were determined at close intervals On the day after the experiment the patient s blood sugar was 340 mg/100 ml and her alkali reserve wa normal

Blood samples for determination of fructose glucose and lactate were taken at short intervals in every experiment In all experiments in diabetic patients and in some of the normal cases the blood pH and standard bicarbonate were determined by the Astrup method (18)

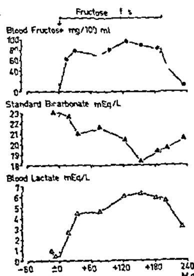


Fig 1 Fructose infusion (1 1 g/kg BW/h) to a normal subject (M V)

Glucose was determined by the o-toluidine method (11) Fructose was determined with meta aminophenol after removal of the glucose with glucose oxidase The details of the method have been published recently (1?) The blood lactate was determined enzymatically with lactic acid dehydrogenase using Boehringer s reagent kit

RESULTS

Healthy subjects (Table I Figs 4 and 5)

In every case the initial blood lactate concentration was below 1 mmol/l During fructose infusion the fructose concentration in blood rose accompanied by a rise in the concentration of lactate In cases in which the pH and standard bicarbonate were also determined a fall in bicarbonate was recorded which corresponded approximately to the rise in lactate (Fig 1)

A rise in blood glucose was noted during fructose infusion in five of the seven cases The greatest rise amounted to 61 mg/100 ml (case E J)

Diabetic patients (Table I)

Before administration of insulin all three patients had a rise in the blood concentration of fructose and lactate in connection with fructose infusion The rise was of the same order of magnitude as when a corresponding amount of fructos was infused in normal subjects (Table I Figs 4 and 5)

In all three cases fructose infu ion was also associated with a considerable rise in blood sugar

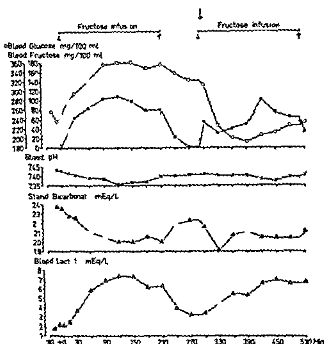


Fig 2 Fructose infusion without and with insulin to the diabetic patient A L ↓ = 20 I U insulin intravenously

Case A L (Fig 2)—who was given a second fructose infusion after i v administration of 20 I U of rapidly acting insulin—had on this occasion as well a rise in blood fructose and lactat which after two hours was only inappreciably smaller than that in connection with the first infusion (without insulin) The rise in both fructose and lactate concentration however seemed to take place more slowly and successively during the second fructose infusion (after administration of insulin)

Case G K (Fig 3) had at the start of the experiment pronounced metabolic acidosis with a standard bicarbonate value of 9 4 mEq/l and blood pH of 7 13 The first fructos infusion had to be discontinued since she showed clinical signs of increasing acidosis and her blood pH fell to 7 05 Insulin and bicarbonate solution had to be administered A steady state infusion of fructose could not be given until she had received repeated intravenous doses of insulin During the later part of the experiment the patient's blood pH and standard bicarbonat rose su cessively as a sign that the keto-acidosis had decreased and the blood sugar had fallen appreciably The blood lactate concentration during fructo e infusion however rose to 8 mEq/l

When the infusion was started case M C had a standard bi arbonate value of 18 mEq/l After

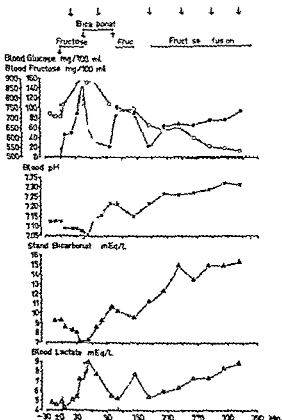


Fig 3 Fructose infusion to the diabeti patient G K At each ↓ 20 I U insulin was given i v For further details see text

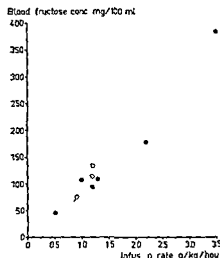


Fig 4 Relation between fructose infusion rate and blood fructose concentration. ● normal subjects ○ diabetic patients

two hours of fructose infusion her standard bicarbonate fell to 12 mEq/l and her blood pH to 7 20

Case A L also had a fall in standard bicarbonate in connection with fructose infusion and an inconsiderable fall in blood pH (Fig 2) The close relation between the blood concentration of fructose and lactate on the one hand and the fall in standard bicarbonate on the other is fully evident from Fig 2

Thus all three patients with diabetes mellitus ad a pronounced increase in blood lactate during ictose infusion of the same ord r of magnitude n healthy subjects The rise in lactate produced i increas in the patients metabolic acidosis resulting in a fall in pH and lactate concentration

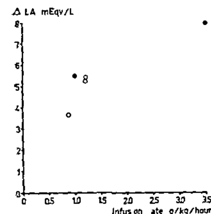


Fig 5 Relation between fructose infusion rate and change in lactic acid concentration (Δ LA) Symbols as in Fig 4

in the blood Fructose infusion was associated with a substantial rise in blood sugar as well When insulin was administered to two of the diabetic patients the blood sugar fell and the standard bicarbonate rose despite fructose being infused Even after administration of insulin a rise took pla e in the blood concentration of fructose and lactate

DISCUSSION

It is known from earlier investigations that fructose is taken up directly in muscles (2) and adipose tissu (7) as well as in the liver (3, 8) In man the uptake of administered fructose into the muscles o curs directly from the blood stream and the glucose taken up is stored in the form of glycogen (2) When fructose is metabolized in the liver the first step is a phosphorylation to fructose I phosphate (4 9) after which there is a splitting into two triose molecules dihydroxyacetone phosphate and glyceraldehyde (9) Glyceraldehyde is further metabolized to lactic acid whereas dihydroxyacetone phosphate can be further metabolized upwards in the Embden-Meyerhof pathway to glucose and glycogen This implies that the rise in blood lactate during infusion of fructose is caused by the liver producing lactate (2 14)

Some relation seems to exist between the amount of fructose supplied per time unit and respectively the fructose concentration in blood and the rise in blood lactate (Figs 4 and 5) At the hi hest rate of infusion (3.5 g/kg BW/h) the rise in lactate was however only inappreciably higher than at the lower rates of infusion indicating that the lactate production approaches a maximum when the fructose supply to the liver increases This suggests that one or several of the enzymes which metabolize fructose in the liver is rate limiting i e proceeds at a maximum at the high fructose concentrations in question here

Our series of diabetic patients is unfortunately too small to permit any conclusions about a possible difference between diabetics without insulin and healthy subjects with respect to lactate production during fructose infusion In our three diab tics the rise in blood lactate nevertheless appeared to be of the same order of magnitude as that in the healthy experimental subj cts Metz et al (15) found on infusion of fructose in diabetic patients

whose insulin had been discontinued for 48–72 h that the formation of lactate was significantly lower than when a corresponding amount of fructose was given to healthy experimental p rsons They however used much smaller doses and shorter infusions than in our experiments

During the initial fructose infusion without insulin all three diabetic patients had a consid rably greater rise in blood sugar than that in the healthy subjects It has recently been demonstrated by means of hepatic vein catheterization in healthy subjects that the glucose production in the splanchnic region falls during fructose infusion (2) Since this occurs despite the liver taking up more fructose than the lactic acid formed it indicates that a consid-rable part of the fructose taken up in the liver is normally stored as glycogen The much greater increase in blood sugar in the diabetic patients indicates that the glucose production in the splanchnic region increases i e the part of the fructose that does not form lactic acid is metabolized to glucose in the liver instead of being stored as glycogen

The rise in lactate which occurred during fructose infusion of 1 g/kg BW/h or more was of sufficient magnitude (5–8 mEq/l) to have a significant effect on the acid base equilibrium so that metabolic acidosis arose The effect was particularly drastic in the diabetic patients M C and G K Even before starting the experiment they had keto acidosis In both cases th re was a pronounced fall in arterial pH in case G K to as low as "04 This patient became greatly affected clinically during fructose infusion and had to be treated acutely with rep ated insulin injections and administration of bicarbonate If fructose infusion had been continued without taking these measures it would presumably have been detrimental to the patient

Thus our results show that rapid administration of fructose can lead to consid rable la tic acid acidosis and—at any rate in patients who already have acidosis—may exacerbate their condition We therefore conclud that rapid infusion of fructose is contraindicat d in acidotic patients The great risk should be stressed esp cially when the patients may b suspected to have an increased produ tion of lactate on grounds of anoxia or for other reasons (10) Andersson et al (1) rec ntly observed two cases of severe acidosis in children which became further exacerbated during fructose ad ministration in one of them with a fatal outcome

In view of the data presented here it is probable that formation of lactic acid during administration of large amounts of fructose did in fact contribute decisively to exacerbating the acidosis and causing the death of one of the children

ACKNOWLEDGEMENTS

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REFERENCES

1 Andersson G Brohult J & Sterner G Increasing metabolic acidosis following fructose infusion in two children. To be published
2 Bergstrom, J & Hultman E Synthesis of muscle glycogen in man after glucose and fructose infusion Acta med scand 18 93 1967
3 Cori C F The fate of sugar in the animal body J biol Chem 70 577 19 6
4 Cori G T Ochoa S Slein M W & Cori C F The metabolism of fructose in liver Isolat on of fructose 1 phosphate and inorganic pyrophosphate Biochim biophys Acta (Amst) 7 304 1951
5 Daughaday W H & Weichselbaum T E Utilization of i v fructose in diabetic acidosis and in pancreatectomized human Metabolism 2 459 1953
6 Drucker W R Miller M Craig J Jefferies W M Levey S & Abbott W E A comparison of the effect of operat on on glucos and fructose metabolism Surg Forum 3 548 1955
7 Froesch E R & Ginsberg J L Fructose metabolism of ad pose tissue 1 Comparison of fructose and glucose metabolism in epididymal adipose tissue of normal rats J biol Chem 237 3317 196
8 Hers H G The conversion of fructose 1 C and sorb tol 1 C to liver and muscle glycogen in the rat J biol Chem 14 373 1955
9 Hers H G & Kusaka T Le metabolisme du fructose 1 phosphate dans le foie B ochim biophys Acta (Amst) 11 4_7 1953
10 Huckabee W E Abnormal resting blood lactate II Lact c ac dosis Amer J Med 30 840 1961
11 Hultman E Rap d specific method for determination of aldosaccha des in body fluids Nature (Lond) 183 108 1959
1 — Studies on muscle metabolism of glycogen and active phosphate in man with special reference to exercise and d et Scand J clin Lab Invest Suppl 94 1967
13 Luke R G D nwoodie A J Linton, A L & Kennedy A C Fructose and glucose tole ance in urem a J Lab cl n Med 64 731 1964
14 Mendeloff A L & We chselbaum T E Role of the human liver in the assimilation of ntravenously adm nistered fructose Metabol sm 2 450 1953

15 Metz, R. Mako M Stevens T & Franklın J The metabolısm of fructose ın diabetes mellıtus J Lab clın. Med 69 494 1967
16 Mıcbon, P., Largan A & Vert P Parenterale Er nahrung mıt Zuckern Nutr et Dıeta (Basel) Suppl 29 1961
17 Miller M., Drucker W R., Owens J E Craıg, J W & Woodward H Metabolısm of ıntravenous fructose and glucose ın normal and dıabetıc subjects J clın. Invest 31 115 1952
18 Siggaard Andersen O Engel K., Jørgensen, K. & Astrup P A micro method for determınatıon of pH carbon dıoxıde tensıon, base excess and standard bıcarbonate ın capillary blood Scand J clın Lab Invest 12 172, 1960
19 Thoren, L. Parenteral nutrıtıon with carbohydrate and alcohol Acta chır scand Suppl 325 75 1964
20 Weıchselbaum T E., Elman R. & Lund R. H Comparatıve utılızatıon of fructose and glucose gıven ıntravenously Proc Soc exp Bıol (N Y) 75 816 1950

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IDIOPATHIC SCOLIOSIS IN OLD AGE

I Respiratory Function[1]

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Abst act Lung function was studi d in a group of 14 female patients between the ages of 57 and 63 who had been diagnosed as idiopathic scoliotics about 50 years ago The examinations comprised spirometry and deter m nation of ventilation and the alveolar gas exchange at rest and during work Roentgenological measurements were used to determine the degree of scoliosis the cases being divided into two groups according to the degree of deformity The moderately deformed group contained seven patients with an angle of less than 60 while the other seven patients made up the severely deformed group with an angle of more than 60 The latter group had significantly lower total and vital capacities maxi mum ventilat on and forced expiratory volume in one second and also a significantly higher respiratory rate and tidal volume The two cases with the most severe degree of scolios s had an impaired alveolar gas ex change the one with the largest angle (102) presenting signs of respiratory insuff ciency with retention of carbon d oxide dur ng oxygen inhalation.

The results thus suggest that the degree of deformity in scolios s is correlated w th lung funct on

Although vital prognosis is said to be impaired by severe scoliosis no detailed analysis of this rela tionship has appeared in the literature Nilsonne and Lundgren (18) have reported however that the mortality in a group of patients with severe idiopathic scoliosis and an observation period of up to 50 years was twice as high as in cor responding groups of the normal population When the study was made the mean age of the living patients was 62 1 years while for the dead patients it was 46 6 years Heart or lung disease was the cause of death in 60 of the latter group whereas almost half of the living patients had physical complaints whi h rendered them incapable of working

The high mortality observed in idiopathic scoliosis and the predominance of heart and lung diseases as the cause of death rais the question of the cardiac and pulmonary function in scoliotic patients and the extent to which the degree of scoliosis is correlated with any impairment of these functions The aim of the present study was to deal with the questions that can be answered by means of data from investigations of lung function

[1] A preliminary report was presented at the annual meeting of the Swedish Med cal Society in 1964

MATERIAL

The s udy was concerned with 15 female patients with idiopath c scolios s who first presented at the Orthopedic Cl nic at Karolinska Institutet during the period 1913–1918 Mean age at examinat on of pulmonary function was 63 years All the patients were among the subjects stud ed by Nilsonne and Lund ren (18) with respect to long term prognos s This larger group however included patients liv ng in distant parts of Sweden for practical reasons the present study was limited by inviting only those patients to part cipate who lived in the Greater Stockholm area A further element of select on was in troduced by some of the patients declining to take part in the study wh ch depended upon voluntary participa tion One of the fifteen patients (no 5) who accepted the inv tation took part only in the Bernstein spirometry All the others completed the examinat ons as planned

METHODS

Stat c lung volume (l BTPS) Total lung capacity and its subdivisions were determined by the hel um dilut on method, using a closed spirometer system *Dynam c l ng volume* (l BTPS) was determined with a modif ed Bern stein Spirometer Normal alues were taken as the data

Table I *Some anthropometric data on 15 patients with scoliosis*

Case no	Age (y)	Height (cm)	Weight (kg)	BMR ( )	W_{130} sitting (kpm/min)	Degree of scoliosis ( )
1	69	165	65	−6	400	43
2	62	164	57	+3	200	67
3	63	150	60	+10	250	97
4	65	155	62	−17	300	10
5	58	150	42	—	300	85
6	57	155	47	−11	300	72
7	63	156	57	±0	450	4
8	63	167	67	+5	400	54
9	65	147	52	−9	300	86
10	64	163	65	+14	300	15
11	57	135	42	+1	200	102
12	63	150	62	+13	200	80
13	65	159	64	−8	300	13
14	67	162	67	+5	350	48
15	63	153	63	+8	250	84
n	15	15	15	14	15	15
$\bar{x}$	63	155 4	59 5	+0 6	300	59 9
S D	±3 4	±8 43	±8 26	±9 51	±75 6	±30 6
Range	57–69	135–167	42–67	−17 – +14	200–450	10–102

n = number of subjects $\bar{x}$ = mean S D = standard deviation

corrected to BTPS presented by Berglund et al (3) Birath et al (5) and Grimby and Soderholm (11) *Distribution of inspired gas* was studied by means of N_2 elimination during oxygen breathing using a Lilly N_2-meter The result was expressed as the time (min) for lowering $F_{E\,N_2}$ to 0 02 Gas distribution was also measured as the helium equilibration time during spirometry *Venous admixture* due to true shunts in the lungs was estimated as outlined by Berggren (2)

Expiratory gas was collected into Douglas bags and the volumes measured with a spirometer Gas samples were analysed for *oxygen and carbon dioxide* by the Haldane technique and for carbon *monoxide* with a hopcalite CO meter (Stålex) as described by Linderholm and Sjostrand (15)

Arterial blood was sampled anaerobically at rest and at the end of the work load period from an indwelling teflon catheter in the brachial artery *Partial pressures of O and CO in arterial blood P O and P CO* mm Hg were measured with a Clark electrode and a Severinghaus P_C-electrode respectively (21) *pH and standard bicarbonate* were determined by the Astrup microtechnique (1) *Carbon monoxide* in blood was determined by the method described by Linderholm et al (16) *Oxygen saturation* of blood *S O*, and *hemoglobin concentration* g/100 ml were measured spectrophotometrically *Dead space* including a correction for breathing valve dead space of 40 ml and 50 ml at rest and during work respectively was calculated from the rearranged Bohr equation *Alveolar oxygen tension P O*, mm Hg was derived from the alveolar air equation assuming arterial CO tension to be equivalent to alveolar CO tension *Diffusing capacity* of the lungs for carbon monoxide $D_{L\,CO}$ ml STPD/min/mm Hg was calculated according to Filley et al (9) with the correction for CO back pressure suggested by Linderholm (14)

Physical working capacity was determined by a test in the sitting position on a bicycle ergometer according to Sjostrand (23) the work capacity being expressed as the effect in kpm/min developed on the ergometer at a heart rate of 130 beats/min (W_{130}) *Electrocardiograms* were recorded by a direct writing ink jet recorder (Mingograph 4_ Elema Schonander Stockholm) at rest in the supine and standing positions during exercise in the sitting position on the bicycle ergometer and $^1/_2$ and 4 min after exercise Leads I II III aVR, aVL aVF CR_1 and V were used at rest during exercise leads CH_4 with the indifferent electrode on the forehead (22)

The *degree of scoliosis* was expressed in terms of the angle formed by the converging limb of the spinal curve according to Cobb (8) The severity of the deformation was classified according to Ponseti and Friedmann (19) and the material was divided into two groups one in which the degree of deformity was from mild to moderate i e less than 60 and the other where it was severe ⩾60

Current statistical methods were used (24)

RESULTS

Anthropometric data on the fifteen scoliotic patients are given in Table I while Tables II and III give the observed and predicted data from the spirometric determinations of lung volumes and ventilatory capacity The patient s actual height was used for the predictions of lung volumes

Table II. Some spirometric data on 14 subjects

Mean values ($\bar{x}$) ± standard deviation (s.d.) and number of individuals (*n*) are given

Case no.	VC (l BTPS)	VC (% of predict.)	TLC (l BTPS)	TLC (% of predict.)	FRC (l BTPS)	FRC (% of predict.)	RV (l BTPS)	RV (% of predict.)	FRC/TLC (%)	FRC/TLC (% of predict.)	RV/TLC (%)	RV/TLC (% of predict.)
1	2 08	68	2 94	63	1 53	63	1 06	65	52	102	36	100
	1 42	44	3 36	54	1 67	65	1 24	80	63	127	48	141
3	1 75	49	2 19	59	1 20	81	0 94	82	54	108	42	140
4	2 53	93	3 49	87	1 57	83	0 99	76	45	88	28	89
5	—	—	—	—	—	—	—	—	—	—	—	—
6	1 86	64	3 37	81	1 80	104	1 64	131	53	108	49	163
7	74	99	4 61	114	2 63	129	1 87	145	57	112	41	178
8	2 38	78	4 59	93	2 45	102	2 01	122	3	106	44	1 6
9	1 83	76	3 01	86	1 30	84	1 18	107	50	98	39	130
10	74	88	4 30	97	2 81	124	1 84	119	62	124	41	121
11	0 68	33	1 33	49	0 69	46	0 65	100	*2	102	49	204
12	1 84	72	2 89	78	1 41	86	1 05	92	49	98	36	120
13	2 66	91	4 12	96	00	96	1 46	103	49	96	35	106
14	2 59	86	3 96	88	2 16	103	1 41	92	55	108	36	103
15	2 10	78	3 27	84	1 46	83	1 17	95	45	90	36	116
n	14	14	14	14	14	14	14	14	14	14	14	14
$\bar{x}$	06	72 8	3 34	80 6	1 77	89 4	1 32	100 6	32 9	104 8	40 0	127 6
s.d.	± 0 63	± 19 6	± 0 96	± 18 4	± 0 58	± 26 6	± 0 40	± 22 5	± 5 66	± 11 2	± 6 1	± 29 1
Range	0 68–2 74	33–99	1 33–4 61	49–114	0 69–2 81	46–129	0 65–2 01	65–145	45–63	96–135	28–49	85–204

VC = vital capacity, TLC = total lung capacity, FRC = functional residual capacity, RV = residual volume

Table III *Ventilatory capacity of 15 subjects*

Case no	MVV_F (l BTPS)	MVV_F (c/min)	MVV_F (of predict)	MVV_{40} (l BTPS)	MVV_{40} (of predict)	$FEV_{1.0}$ (l, BTPS)	$FEV_{1.0}$ (of predict)	FEV ( )	FEV (of predict.)
1	57	114	61	39	50	1 43	65	68	92
2	41	76	41	28	34	1 15	48	81	107
3	26	92	27	29	35	0 87	45	73	96
4	47	84	48	31	39	1 65	81	65	87
5	32	109	31	20	24	0 82	39	60	78
6	45	77	43	43	49	1 53	67	8	106
7	66	90	67	45	55	1 75	83	67	88
8	73	94	75	43	52	1 61	66	72	95
9	22	83	23	28	35	1 47	81	82	109
10	45	84	46	47	58	2 03	88	74	99
11	13	67	13	1	14	0 30	18	5[illegible]	68
12	57	12[illegible]	58	39	48	1 21	62	68	89
13	83	78	86	69	86	1 84	86	71	95
14	29	80	31	27	34	1 95	90	76	101
15	35	75	36	40	49	1 51	74	73	96
n	15	15	15	15	15	15	15	15	15
$\bar{x}$	44 7	88	46	36 0	44	1 41	66	71	94
S D	±19 7	±15 6	±20 4	±13 5	±16 8	±0 47	±20 8	±8 [illegible]	±10 9
Range	13–83	67–1[illegible]	13 86	12–69	14–86	0 30–2 03	18–90	52–8[illegible]	[illegible]8–10[illegible]

MVV_F MVV_{40} = maximum voluntary ventilation at free re piratory rate and at 40 brea hs/min $FEV_{1.0}$ = forced expiratory volume in one second FEV = $FEV_{1.0}$ expressed as percentage of vital capacity

Spirometry

In keeping with previous studies (4 6 7 10 12 13 17) the total and vital capacities—and hence to some extent the functional residual capacity —were smaller than predicted whereas the re sidual volume was essentially normal Since the [illegible] ute values for the residual volume were [illegible] its relative measure—expressed as RV/TLC (residual volume/total lung capacity)—was elevated though this is not to be regarded as a sign of obstructive lung disease in the cases under review The ventilatory capacities (Table III) that are dependent on both time and volume ($MVV_{F\,re}$ MVV_{40} and $FEV_{1.0}$) were similarly depressed whereas FEV% (which is independent of the volume) showed ordinary values indicating that the flow was normal

Certain significant differences in lung volumes and ventilatory capacities were found between the group with moderate scoliosis (10 –60) and the group with a severe deformity (60 –102) cf Figs 1 and 2 The former group had higher means for vital capacity and total lung capacity expressed as percentages of the predicted values ($P<0.01$ and $P<0.05$ respectively) while RV/

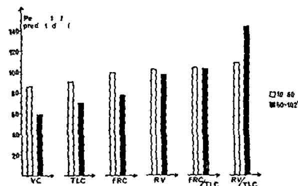


Fig 1 Lung volumes in per cent of predicted value of the two deformity groups Symbols as in Table II □ 10–60 ■ 60–10

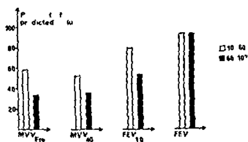


Fig 2 Ventilatory capacities in per cent of predicted value of the two deformity groups Symbols as in Table III □ 10–60 ■ 60–102

TLC wa higher for the group with a severe deformity ($P < 0.05$) Similarly the group with a moderate degree of scoliosis had higher means for maximum voluntary ventilation at free respiratory rate and at 40 breaths/min as well as for forced expiratory volume in one second ($P < 0.05$ in each case)

Ventilation and alveolar gas exchange

Data obtained at rest and during exercise are given in Table IV The load used averaged 86 % of the physical working capacity at a heart rate of 130 beats/min (W_{130}) and the pulse rates were 90% of the highest level recorded during the previous test on a bicycle ergometer in the sitting position No significant differences were found between the two scoliotic groups in the degree of relative circulatory load expressed as percentage of W_{130} or of the highest heart rate recorded previously but certain differences in the adaptation of ventilatory function were significant

For the group with a severe deformity the mean total ventilatory minute volume amounted to 72 of th maximum voluntary ventilation (MVV) measured during spirometry while the corresponding mean for the group with a moderate deformity amounted to 45% ($P < 0.05$)

The greater ventilatory load for the severe scoliotics is reflected by higher respiratory rates ($P < 0.05$) and smaller tidal volumes ($P < 0.05$) compared with the group with moderate deformities Shallow rapid breathing usually involves elevated ventilation of dead space with an increased V_D/V_T quotient 0.30 being regarded as the normal upper limit This value was either reached or exceeded in seven out of fourteen observations during work but there was no difference between the two groups The ventilatory equivalent for oxygen—V_E/V_{O_2} i e the ventilation p r litre oxygen uptake—amounted to 28 and 27 litres for the groups with severe and moderate deformity respectively both these values lie within the normal variation

The alveolar gas exchange was impaired in two cases (nos 3 and 11) in which the diffusion capacity ($D_{L\,CO}$) and the arterial oxygen tension (P_{aO_2}) were depressed and the arterial carbon dioxide tension (P_{aCO_2}) somewhat elevated indicating a slight carbon dioxide retention These cases also had the most severe thoracic deformities 97 and 102 according to Cobb The corresponding means for the two groups did not differ significantly either from each other or from pertinent normal data

The alveolar arterial oxygen pressure difference ($P_A - P_aO$) was higher in most cases than the level of 10–20 mm Hg that most authors regard as the normal upper limit There was no significant difference between the group means

The distribution of inhaled gas determined by th nitrogen elimination time was normal in th nine cases in which it was studied

The calculated intrapulmonary shunt (Q shunt/Q total) exceeded in many cases 6 to 7%, the figure given as the normal upper limit Assuming that the nitrogen elimination time was normal in the cases in which it was not determined the results indicate that the majority of the fourteen scoliotic patients had elevated shunt values There was no significant difference between the group means Case no 11 in which the diffusion capacity wa impaired had a pathologically high P_{aCO_2} during oxygen inhalation the pattern being that of a patient with pulmonary insufficiency who retains carbon dioxide when the hypoxaemic drive on the respiratory centr is eliminated

DISCUSSION

The value of the present results for assessing cases of idiopathic scoliosis is somewhat curtailed by th positive selection which took place cf Nilsonne and Lundgren (18) Normal data for the corresponding decades and sex are not available which to some extent makes comparisons uncertain On the other hand cases with these observation p riods have not been published in the literature

The patient s actual height was used for calculating the predicted spirometric values It would be wrong to make a correction for the patient s reduced height due to the deformity because as pointed out by Bergofsky et al (4) it is not possible to estimate the expected normal height for these subjects It should be considered that in a corresponding female material a difference of 10 cm in height affected the calculation for instance of the vital and the total capacities by 0.41 and 0.67 l respectively It should also be noted that the use of the actual height b

Table IV *Ventilation and alveolar gas exchange at rest and during exercise in 14 subjects*

Subj no	Load (kpm/min)	Ventilation (l BTPS/min)	Resp rate (c/min)	Tidal vol (l BTPS)	$\frac{V_D}{V_T}$	$\frac{V_E}{V_{O_2}}$	Oxygen uptake (ml STPD/min)	Heart rate (beats/min)
1	Rest		13					68
	300	27 9	24	1 16	0 31	28 6	973	128
2	Rest		39					64
	200	30 0	50	0 60	0 39	37 5	801	1?8
3	Rest		18					80
	200	31 0	45	0 69	0 37	28 0	1108	155
4	Rest		21					6?
	150	22 6	22	1 03	0 38	37 1	609	99
6	Rest		18					—
	300	23 4	24	1 10	0 ?5	29 3	901	145
7	Rest		16					56
	400	?8 5	18	1 58	0 21	26 4	1084	140
8	Rest		—					70
	300	?4 4	18	1 36	0 30	26 7	914	114
9	Rest		—					58
	200	21 6	32	0 68	0 22	27 7	779	112
10	Rest		—					68
	200	18 8	27	0 70	0 35	23 9	785	1?5
11	Rest	3 9	17	0 23	0 25	25 5	154	—
	100	10 5	22	0 48	0 3?	19 8	529	—
12	Rest	5 7	13	0 44	0 29	27 0	210	—
	300	26 5	24	1 11	0 23	26 0	1020	144
13	Rest	5 1	17	0 30	0 33	28 8	178	—
	300	27 7	18	1 54	0 23	25 7	1075	—
14	Rest	6 1	19	0 32	0 37	27 4	?24	7?
	300	?1 7	20	1 08	0 14	21 8	996	150
15	Rest	8 0	17	0 47	0 35	33 5	239	72
	300	29 1	23	1 27	0 13	29 9	974	1 0

V_D = dead space V_T = tidal volume V_E = total ventilation V_O = oxygen uptake P = pressure a = arterial A = alveolar Stbk = standard bicarbonate Q = blood flow

[a] After 10 O_2 inhalation [b] After 5 O inhalation

sides involving an underestimation of the calculated volumes also leads to an underestimation of the reductions observed in the lung volumes

The reduction of the static lung volumes and the ventilatory capacities that depend on volume was more marked for the group with severe orthopedic deformities and indicates a restriction of the lung function Restriction in this sense includes changes in the lung parenchyma and the chest wall as well as in the respiratory muscles However the normal absolute values for the residual volumes suggest that the lung tissue itself had not undergone restrictive changes

On the other hand the abnormal relationship between respiratory volume and vital capacity indicates an impairment of the chest bellows function Measurements of work of breathing in a body respirator have been reported by Bergofsky et al (4) who found that when scoliotic patients were ventilated with fractions of the vital capacity similar to those of normal cases the work of the chest bellows was five times as great The increased work of breathing with the normal quotient between tidal volume and vital capacity indicates that this increment is dependent not only upon the size but also upon the deformity of the thorax In the present study the relationship between tidal volume during work and vital capacity did not differ between the severe and moderate groups indicating that the patients

						During inhalation 100 O_2			
P_{a} o (mm Hg)	P co (mm Hg)	pH	Stbk (mEq/l)	Diff cap (ml STPD/min) (mm Hg)	$(P_A-P)O_2$ (mm Hg)	Nitrogen elimination (time min)	Q shunt / Q total	P o (mm Hg)	P_{a} co (mm Hg)
79	36	7 43	[illegible]4						
7[illegible]	43	7 33	[illegible]0	14 7	3[illegible]	[illegible] 83	0 083	563	29
77	39	7 41	24						
81	39	7 39	22	14 4	25	45	0 065	584	35
74	43	7 42	25						
67	48	7 37	20	9 5	8	2 17	0 059	583	44
82	37	7 46	24						
96	35	7 44	[illegible]3	16 [illegible]	15	2 62	0 043	633	29
80	43	7 37	[illegible]3						
94	39	7 37	[illegible]1	17 1	13	1 18	0 063	595	38
86	37	7 46	23						
93	40	7 35	[illegible]0	26 4	14	3 50	0 063	590	38
89	35	7 43	23						
92	46	7 37	22	32 6	8	1 50	0 091	549	34
83	33	7 40	23						
86	39	7 34	22	14 2	20	2 67	0 070	578	37
98	37	7 44	25						
93	44	7 37	[illegible]3	24 1	2	1 67	0 038	6[illegible]4	35
68	45	—	—		[illegible]8				
56	46	7 39	—	7 9	26	—	0 101[a]	5 [illegible]	55
67	39	7 43	—		34				
73	38	7 37	—	21 4	33	—	0 103[a]	528	41
74	38	7 42	25		23				
82	34	7 41	22	19 8	24	—	0 117[a]	504	37
74	43	7 4	24		14				
82	46	7 37	22	24 0	15	—	0 160[a]	408	4[illegible]
81	37	7 45	—		23				
89	35	7 41	—	20 0	17	—	0 107[b]	510	36

adapt to the increased breathing work Above a certain limit as in the two most severely deformed patients an increased load on the chest bellows in connection with muscular exercise upsets the normal alveolar gas exchange and results in an alveolar hypoventilation with carbon dioxide re tention and hypoxaemia The reduced diffusion capacity may thus be a consequence of an alveolar hypoventilation with a functional" reduction of the surface area for gas exchange

Previous bronchospirometric studies (25) have shown that gas exchange is impaired in the region of the lung on the concave side of the curvature The findings of elevated valu s of alveolar arte rial oxygen pressure gradients—as in a disturbed ventilation/perfusion relationship—and of "phy siological shunts are consistent with regional functional disturbances

The demonstrated relation between the skeletal deformity and the disturbance of lung function agrees with previous reports (4 6 17 20) The functional importance of th skeletal deformity is well illustrated by the finding that the case with the most severe scoliosis 102 developed signs of pulmonary insufficien y during the inhalation of 100 per cent oxygen emphasizing the clinical significance of making a thorough examination of the lung function in scoliotic patients

REFERENCES

1 Andersen, O S Engel K Jorgensen, K & Astrup P A micro method for determination of pH carbon dioxide tension, base excess and standard bicarbonate in capillary blood Scand J Clin Lab Invest 1 172, 1960

2 Berggren S M The oxygen deficit of arterial blood caused by non ventilating parts of the lung Acta physiol scand Suppl 11 194?
3 Berglund E Birath G Bjure J Grimby G Kjellmer I Sandqvist L & Soderholm B Spirometric studies in normal subjects I Forced expirograms in subjects between 7 and 70 years of age Acta med scand 173 185 1963
4 Bergofsky E H Turino G M & Fishman A P Cardiorespiratory failure in kyphoscoliosis Medicine (Baltimore) 38 263 1959
5 Birath G Kjllmer I & Sandqvist L Spirometric studies in normal subjects II Ventilatory capacity tests in adults Acta med scand 173 193 1963
6 Caro C G & DuBois A B Pulmonary function in kyphoscoliosis Thorax 16 282 1961
7 Chapman E M Dill D B & Graybiel A The decrease in functional capacity of the lungs and heart resulting from deformities of the chest Pul monocardiac failure Medicine (Baltimore) 18 167 1939
8 Cobb J R Outline for the study of scoliosis Instr Course Lectures Amer Acad of Orthop Surg 5 61 1948
9 Filley B F MacIntosh D J & Wright G W Carbon monoxide uptake and pulmonary diffusion capacity in normal subjects at rest and during exer cise J clin Invest 33 530 1954
10 Flagstad A & Kollman S Vital capacity and muscle study in 100 cases of scoliosis J Bone Jt Surg 1026 7_4 1928
11 Grimby G & Soderholm B Spirometric studies in normal subjects III Static lung volumes and maxi mum voluntary ventilation in adults with a note on physical fitness Acta med scand 173 199 1963
12 Gucker T Changes in vital capacity in scoliosis J Bone Jt Surg 44 469 196?
13 Iticovici H & Lyons H Ventilatory and lung volume determinations in patients with chest deformi ties Amer J med Sci ?3_ 265 1956
14 Linderholm H On the significance of CO tension in pulmonary capillary blood for determination of pulmonary diffusing capacity with the steady state CO method Acta med scand 156 413 19?7
15 Linderholm H & Sjostrand T Determination of carbon monoxide in small gas volumes Acta physiol scand 37 _40 1956
16 Linderholm H Sjostrand T & Soderstrom, B A method for determination of low carbon monoxide concentration in blood Acta physiol scand 66 1 1966
17 Mankin H J Graham J J & Schack J Cardio-pulmonary function in mild and moderate idiopathic scoliosis J Bone Jt Surg 46 A 53 1964
18 Nilsonne U & Lundgren K D Long term prog nosis in idiopathic scoliosis Acta orthop scand In print
19 Ponseti, I V & Friedmann B Prognosis in idio-pathic scoliosis J Bone Jt Surg 32 A 383 1950
20 Schaub V Buhlman A & Kalin R Das Kypho-scoliosehetz und seine Pathogenese Cardiologia (Ba sel) 4 147 1954
21 Severinghaus J W & Bradley A F Electrodes for blood pO and pCO determination J appl Physiol 13 515 1958
22 Sjostrand T The electrocardiographic work and hypoxemia tests Scand J clin Lab Invest 3 1 1951
23 — Clinical physiology pp 515–530 Scandinavian University Books Stockholm 1967
24 Snedecor G W Statistical methods Iowa State Col lege Press Iowa 1959
25 Steinmann E P Die Funktionsprufung der einzelnen Lunge bei der Kyphoskoliose Z Orthop 80 ?0 1951

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CEREBRAL BLOOD FLOW AND OXYGEN CONSUMPTION IN BARBITURATE POISONING

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Abstract Cerebral blood flow (CBF) and cerebral metabolic rate of oxygen ($CMRo_2$) have been determined by the nitrous oxide method in five cases of severe barbiturate poisoning (blood barbiturate level 2.2–15.4 mg/100 ml) All cases needed prolonged artificial ventilation (IPPV) to survive Mean CBF was 30±9 ml/100 g min and mean $CMRo_2$ was 1 5±0 4 ml/100 g min Within the temperature range studied 31 7 C–37 8 C body temperature (BT) was correlated to $CMRo_2$ according to the equation $BT = 6\,6 \times CMRo_2 + 25$ the correlation coefficient being 0 96 ($p<0\,01$) A similar correlation was found between CBF and BT ($r=0\,93$ $p<0\,05$) The cerebral arteriovenous oxygen difference was normal and rather constant in spite of generally low values for arterial Pco_2 There was no indication of inadequate oxygen supply to the brain

The mortality rate in barbiturate poisoning has been markedly reduced during the last decade (2 9 12 16) However owing to the large number of poisonings certain complications chiefly pulmonary oedema and persisting cerebral injuries are seen in many patients The treatment of these complications may be difficult and the underlying mechanisms are but incompletely known (6 7)

A barbiturate poisoning is characterized by among other symptoms hypothermia In the most severe cases body temperature descends below 30 C The barbiturates inhibit certain steps of the respiratory chain in the cells (8 11) thereby decreasing metabolism and oxygen consumption In addition hypothermia reduces the metabolism and oxygen consumption (1 8) Gleichmann et al (3) in 1962 demonstrated a connection between depth of narcosis as determined by EEG and reduction in cerebral cortical oxygen consumption in dogs However there are as yet only a few investigations reporting data on cerebral blood flow (CBF) and cerebral metabolic rate of oxygen (CMR_{O_2}) in barbiturate poisoning in man Gottstein et al (4) determined CBF and CMR_{O_2} in seven patients suffering from superficial barbiturate induced coma None of their cases needed artificial ventilation CMR_O was reduced by 35–70° These extremely low values were normalized following administration of analeptic drugs These authors presume that nerve cells die when oxygen consumption decreases below 20 % of the normal value This is their justification for using central stimulating drugs in the treatment of barbiturate poisoning However vast clinical experience has eliminated this kind of drugs from the *therapeutic arsenal in Scandinavia* (2 9 12 16) Hence it was considered of interest to determine CBF and CMR_O quantitatively in severe cases of barbiturate poisonings treated according to The Scandinavian Method Especially the relation between CMR_O and body temperature was considered to be of interest in order among other reasons to further elucidate the prolonged time of cerebral survival during circulatory arrest and a possible cerebral genesis of the pulmonary oedema In this preliminary study CBF and CMR_O have been measured in a small material without the above mentioned complications in order to provide basic values for further investigations in progress

MATERIAL

The patients were selected for these studies according to the following criteria (*a*) Deep coma with hypothermia and need for prolonged artificial ventilation caused by proven barbiturate poisoning. (*b*) Body temperature slowly increasing at the moment of measurement but not exceeding 38 C The reason for this limit was that, after passing 38 C the patients usually return gradually to consciousness (*c*) The patients had to be rather stable with respect to body temperature which was not allowed to change more than 0 2 C one hour before and one hour after the measurement of cerebral blood flow In the original material one patient was excluded because the temperature rose 2 0 C in one hour during and after the measurement (*d*) No history or signs

Table I *Clinical observations in five patients unconscious due to barbiturate poisoning*

Pat no	Age	Sex	Type of barbiturate	Blood conc of barb [a] (mg/100 ml)	Duration of coma (h)	Duration of resp treatm (h)	Lowest temp (C)	Temp at CBF measurement (C)	Dose of nor epinephrine (μg/min)
1	37	♂	Comb [b]	12 3	48	24	35 1	37 8	6
2	29	♀	Comb [b]	15 2	52	38	33 9	34 7	12
3	33	♀	Comb [b]	9 0	20	12	34 3	34 3	9
4	59	♀	Shortacting	2 2	70	65	30 7	31 7	6
5	56	♀	Comb [b]	10 0	42	16	33 5	37 2	6

[a] Calculated as sodium phenobarbital
[b] Combination of short and long acting barbiturate

of cardiac or cerebro vascular disease (*e*) Criteria *a* to *d* had to coincide at a time when clinical and laboratory facilities were available

The relevant data about the five patients and their clinical condition is summarized in Table I They were deeply un conscious because of attempted suicide with different barbi turates They were artificially ventilated (IPPV) and received intravenous infusion with Ringer glucose solution about 2000 ml/24 h All patients on admission had low arterial blood pressure which was effectively treated by norepineph rine administration (6–12 μg/min) The systolic blood pressure was kept constant at 100 to 130 mm of mercury All the patients had a period of hypothermia Analyses of barbiturate concentration in blood were made on admission and revealed levels of 2 2–15 2 mg/100 ml blood calculated as phenobarbital A screening procedure was used to exclude the presence of other poisons in blood and urine All the patients survived the poisoning without complications

METHOD

Cerebral blood flow (CBF) and *cerebral metabolic rate of oxygen* ($CMRo_2$) were determined by the nitrous oxide method of Kety and Schmidt (5) Arterial blood samples were col lected from teflon catheters percutaneously introduced into the brachial or radial artery The left jugular bulb was punctured with a hypodermic needle Five pairs of arterial and jugular venous blood samples were taken at appropriate intervals during a measuring period of 15 min during which 15 of nitrous oxide was added to the inspired air via the Engstom respirator Samples for analysis of oxygen content were taken immediately before the administration of the nitrous oxide mixture Samples for determination of arterial Po_2 and Pco_2 were taken at intervals before during and after the CBF measurement All blood samples were taken in 10 ml siliconized all glass syringes The dead space in the syringes was filled with 5 heparin solution The volume of the dead space was determined and the values for nitrous oxide and oxygen content were corrected for dilution by the heparin

Blood analysis The nitrous oxide content was analysed in duplicate by the method of Orcutt and Waters (10) with the slight modification introduced by Kety Oxygen content was determined in duplicate by the Van Slyke Neill manometric technique (17) The oxygen analyses were done immediately after sampling Arterial oxygen tension (Po_2) and carbon dioxide tension (Pco_2) were measured with a macro oxygen electrode (Beckman) and a Severinghaus electrode respectively mounted in a Beckman Modular cuvette The readings were made directly on a Beckman physiological gas analyser after calibration with analysed gas mixtures The measurements were made at 37 C The

Table II *Cerebral blood flow and oxygen metabolism in five patients deeply unconscious due to barbiturate poisoning*

Pat no	CBF (ml/100 g/min)	$(A-V)o_2$ (vol)	$CMRo$ (ml/100 g/min)	Temp [a] (C)	P_{ao} [b] (mm Hg)	P co [c] (mm Hg)	BP [d] (mm Hg)
1	41	4 7	1 9	37 8	60	28	110/90
2	23	6 3	1 4	34 7	55	26	120/90
3	27	6 0	1 6	34 3	89	33	110 85
4	21	4 7	1 0	31 7	73	32	130 90
5	36	4 9	1 8	37 2		25	110 80
Mean ± S D	30±9	5 3±0 8	1 5±0 4			29±4	

[a] Rectal temperature during CBF measurement
[b] Arterial Po corrected to body temperature
Arterial Pco corrected to body temperature
[d] Riva Rocci method

values were corrected to the body temperature according to the Severinghaus blood gas calculator (Radiometer)

Statistical methods The statistical calculations were performed according to Snedecor (13) The following probability (*p*) levels of significance were used $p<0.001$ highly significant $p<0.01$ significant and $p<0.05$ probably significant

RESULTS

The values for CBF cerebral arterio venous oxygen difference $(A-V)_{O_2}$ CMR_O body temperature at CBF measurement arterial P_O arterial P_{CO} and arterial blood pressure are presented in Table II

From the arterial P_{CO} values it can be seen that the patients have been hyperventilated Mean arterial P_{CO_2} was 29 ± 4 mm Hg Despite this there were generally low values of arterial P_{O_2} which is common in unconscious patients being artificially ventilated with air However the prevailing oxygen tension corresponded to oxygen saturation values in the range 90–96 % and the oxygen available to the brain was hence satisfactory since the arterial blood pressure was kept constant

At the time of the CBF measurement three of the patients had hypothermia the others had normal body temperature

Cerebral blood flow All cases had values lower than those given by Kety and Schmidt (5) for healthy conscious individuals Except in case 1 the values may be regarded as very low In the three cases with hypothermia the values were extremely low The mean value for the material was 30 ± 9 ml/100 g/min

Cerebral $(A-V)_O$ was normal or low and rather constant in all five cases mean value 5.3 ± 0.8 vol %

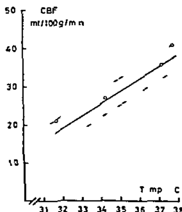


Fig 1 The relation between CBF and body temperature The regression line $\pm$ S D is shown. The equation for the regression line is $y = 3.5 - 85$ S.D $= 3.4$ $r = 0.93$ $(p<0.05)$

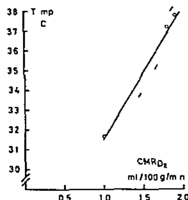


Fig 2 The relation between body temperature and CMR_O The regression line $\pm$ S D is shown. The equation for the regression line is $y = 6.6x + 25$ S D $= 0.8$ $r = 0.96$ $(p<0.01)$

Cerebral metabolic rate of oxygen was reduced in all cases In case 4 the extremely low value of 1 0 ml/100 g/min was found. The mean value was 1.5 ± 0.4 ml/100 g/min

Correlations The relation between CBF and body temperature is shown in Fig 1 CBF was probably significantly correlated to body temperature ($r = 0.93$ $p<0.05$)

The relation between body temperature and CMR_O is shown in Fig 2 Body temperature was significantly correlated to CMR_O ($r = 0.96$ $p<0.01$) No correlation was found between CBF and arterial P_{CO} in these cases

DISCUSSION

In barbiturate induced deep coma in man there was found a considerable reduction in cerebral oxygen consumption as an expression of the reduced metabolism in the brain tissue The reduction amounted to about 50 % in the whole material The case with the lowest temperature had a reduction of about 70 Of special interest is the linear correlation between cerebral oxygen consumption and body temperature ($r = 0.96$ $p<0.01$) Of course this correlation holds only for the temperature range of the material 31 7–37 8 C

The degree of reduction in cerebral oxygen consumption in these cases is higher than the reduction observed during induced hypothermia combined with the general anesthesia Most authors (1 8) report a reduction of about 50 for a 10 C lowering of the body temperature In the present investiga

tion there was a reduction of 50% already at a body temperature of about 35 C i e two degrees below normal This big difference between the two conditions is explained by the fact that in the presented material body temperature sunk mainly as a result of reduced metabolism and heat production and not as a result of active cooling

Bering working with monkeys in induced hypothermia found a linear correlation between log CMR_O and the reciprocal of the absolute temperature (1) This was true for the temperature range of 15–37 C A close inspection of his diagrams reveals a near linear correlation between temperature and CMR_O in the range of 32–37 C Thus the results in man so far agree with those in monkey

In addition there was found a linear correlation between cerebral blood flow and body temperature which was probably significant ($r = 0.93$ $p \sim 0.05$) There was no correlation at all between arterial P_{CO} and CBF or the cerebral $(A–V)_O$ The latter was rather constant in all cases despite big differences in arterial P_{CO} These results suggest that cerebral blood flow follows the metabolic demands of the brain tissue despite big variations in arterial P_{CO} Definite conclusions in this respect necessitate further studies

Judged from the normal or low cerebral $(A–V)_O$ there was no indication of inadequate blood supply to the brain as a whole However there may be smaller parts of the brain with inadequate oxygen supply in spite of a normal $(A–V)_O$

The results presented in this work do not support the opinion of Gottstein (4) who recommended the use of analeptic drugs in treatment of these cases According to him nerve cells die when oxygen consumption is reduced below 20° of the normal but he does not give any reason for his opinion The lowering of the oxygen demands of the brain ought to be a protection against anoxic brain injuries in these cases The use of analeptic drugs in treatment of barbiturate poisonings increases the oxygen demand in the nerve cells which may be dangerous in these conditions with reduced cerebral blood flow and disturbed regulation of cerebral circulation

It is well known that in hypothermia the brain tissue survives circulatory arrest longer than in normothermia The time interval between the cessation of circulation and the development of irreversible brain injury depends on the degree of oxygenation at the moment of circulatory arrest (15) But also the rate of cerebral oxygen consumption determines the rate of lowering of the cerebral oxygen tension and thereby the development of brain injury It is a common clinical impression that the results of cardiac resuscitation are more successful in hypnotic poisoning than in other conditions in other words the time interval seems to be prolonged Of course it is not possible to predict how many minutes of cardiac arrest the brain will survive in hypnotic poisonings because we do not know the degree of oxygenation at the moment of cessation of circulation On the other hand it has been shown that the amount of ATP is increased in nerve cells during barbiturate narcosis (8 14)

ACKNOWLEDGEMENTS

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REFERENCES

1 Bering E A Jr Effect of body temperature change on cerebral oxygen consumption of the intact monkey Amer J Physiol 200 417 1961
2 Clemmesen C New line of treatment in barbiturate poisoning Acta med scand 148 83 1954
3 Gleichmann U Ingvar D H Lassen N A Lubbers D W Siesjo B K & Thews G Regional cerebral *cortical metabolic rate of oxygen and carbon dioxide* related to the EEG in anesthetized dog Acta physiol scand 5 82 1962
4 Gottstein O Bernsmeier A Lehn H & Niedermayer W Hämodynamik und Stoffwechsel des Gehirns bei Schlafmittelvergiftungen Dtsch med Wschr 86 2170 1961
5 Kety S S & Schmidt C F The nitrous oxide method for the quantitative determination of cerebral blood flow in man theory procedure and normal values J clin Invest 27 476 1948
6 Lassen N A Helsborg H C & Vangtorp A Lunge odem ved antichockbehandling af svære narkotiske forgiftninger Ugeskr Læg. 124 825 1962
7 Matell G The effect of ethacrynic acid as a treatment for pulmonary congestion and oedema in intoxications with hypnotics Opusc med (Stockh) 10 261 1965
8 Nesbakken R Det biokjemiske grunnlag for den kliniske brug av hypotermi Nord Med 72 1279 1964
9 Nilsson E On treatment of barbiturate poisoning Acta med scand Suppl 253 127 1951
10 Orcutt F S & Waters R M A method for the determination of cyclopropane ethylene and nitrous oxide in blood with the Van Slyke Neill manometric apparatus J biol Chem 117 509 1937
11 Paleus S Myocardiets metabolism Nord Med 75 4 1966

1? Reis G von Behandlingen av akuta forgiftningar med somnmedel och narcotica. Svenska Läk Tidn 57 1885 1960
13 Snedecor G W Statistical methods Iowa State College Press Ames Iowa 1956
14 Stone W E Acid soluble phosphorus compounds and lactic acid in the bra n J biol Chem 135 43 1940
15 Sugioka K McKnight R & Davis D Brain tissue oxygenation before during and after simulated cardiac arrest Physiologist 3 156 1960
16 Thorstrand C A ten year study of the mortality rate due to hypnoti and sedative intoxications Opusc med (Stockh) 10 270 1965
17 Van Slyke D D & Neill J M The determination of gases in blood and of solutions by vacuum extraction and manometric measurement J biol Chem 61 5 3 19 4

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CORTICOSTEROIDOGENIC EFFECT OF LONG ACTING BETA$^{1-24}$CORTICOTROPHIN (CIBA 42 915 BA)

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Abstract Synthetic beta$^{1-24}$corticotrophin, synthesized by Kappeler and Schwyzer has been modified by the manufacturers by adsorbing it onto zinc phosphate The adrenal cortex action of this preparation has been evaluated in eight non-endocrine patients A prolonged corticosteroidogenic effect is found as reflected by the increase of plasma corticosteroids (cortisol + corticosterone) and urinary 17 ketogenic steroids One mg of the modified beta$^{1-24}$corticotrophin (Ciba 42.915 Ba) injected i.m maintained significantly elevated plasma corticosteroids for at least 36 hours.

Corticotrophin (ACTH) from sheep pig and man is a straight chain polypeptide containing 39 amino acids (4 23 32) Specific species variation is only found in amino acids 25–33 (24)

From the study of fragments obtained by hydrolysis of highly purified natural ACTH as well as by synthesis it can be seen that only the first N terminal 19–24 amino acids are necessary to obtain organotrophic activity of the same range as that of natural ACTH (15 16 25)

In 1961 Kappeler and Schwyzer (19) synthesized beta$^{1-24}$corticotrophin with an amino acid sequence identical with N terminal 1–24 amino acids in natural ACTH and in 1963 Schwyzer and Sieber (31) reported on the total synthesis of beta corticotrophin with an amino acid sequence characteristic of the porcine species

In 1962 a third international standard for corticotrophin was introduced (2) The biological activities of synthetic beta$^{1-24}$corticotrophin and of synthetic porcine beta-corticotrophin have been compared with this standard Synthetic beta$^{1-24}$corticotrophin has a biological activity equivalent to 106 units/mg (30) Comparative experiments in man reveal that there is no significant difference in the effect of natural ACTH and of beta$^{1-24}$corticotrophin on urinary 17 ketogenic steroids (17 KGS) 17 ketosteroids (17 KS) and plasma corticosteroids (18 20 21 22) Synthetic porcine beta corticotrophin has a biological activity equivalent to 115 units/mg (3)

Beta$^{1-24}$corticotrophin has been used to test adrenocortical responsiveness (1 18 26 33) However since its action is of short duration (22 33) it has little therapeutical use

The manufacturers have modified beta$^{1-24}$corticotrophin by adsorbing it onto zinc phosphate It has been shown that this preparation has a prolonged action (5 13) The present paper reports on the adrenal cortex action of this preparation in eight patients The preparation (Ciba) will be marketed under the name Synacthen Depot®

MATERIAL

The material consisted of eight patients (from Diakonissestiftelsen, Medical Dept. (Head T Hilden M D)) The investigations were carried out while the patients were convalescents There were no signs of endocrine disorders kidney or liver diseases. None of the patients had been given or was under treatment with ACTH or adrenal cortex steroids. All patients were of normal weight

METHODS

Each patient received 1 mg Ciba 42.915 Ba i.m at 8 a.m Plasma corticosteroids were estimated at 8 a.m 4 p.m and 11 p.m on the day prior to treatment, immediately before injection and 4 8 12 16 24 and 36 hours later

All blood samples were drawn in heparinized glass tubes by venepuncture Plasma was separated on the same day and stored deep frozen until analysis.

Unconjugated corticosteroids (cortisol + corticosterone) in plasma were determined by a fluorimetric method slightly modified (28) in accordance with de Moor and Steeno (27) 24-hour urine specimens were collected on the day before the day of and the day after injection 17 KGS were estimated on the 24-hour urine specimens (9)

Table I *Plasma corticosteroids and urinary 17 keto ic steroids on the day before the day of and the day after i m injection of 1 mg Ciba 42 915 Ba*

Case no	Sex	Age (y)	Plasma corticosteroids (μg/100 ml) First day 8 a m	4 p m	11 p m	Second day 8 a m	12 a m	4 p m	8 p m	12 p m	Third day 8 a m	8 p m	Urinary 17 KGS (mg/24 hour) Day 1	Day 2	Day 3
1	♀	55	21 5	10 1	8 7	12 2	51 0	57 2	54 8	52 7	41 7	21 3	17 0	76 0	44 5
2	♀	75	18 3	9 1	5 8	16 6	49 3	55 8	5 5	62 3	63 4	31 6	5 9	64	73 2
3	♀	63	13	10 0	8 4	25 7	67 6	74 2	75 5	65 8	39 9	10 0	8 4	55 7	20 ?
4	♀	49	19 9	10 6	4 5	13 0	40 4	58 2	56 0	57 5	55 7	37 4	4 7	43 1	35 8
5	♀	49	12 8	11 7	6 8	15 8	41 8	43 5	38 0	20 8	18 7	9 1	6 2	26 7	9 6
6	♀	78	15 1	12 9	3 8	15 1	50 3	49 3	58 5	58 8	71 8	55 8	6 3	43 3	83 3
7	♂	73	14 7	13 5	9 7	13 8	49 8	56 0	59 2	58 6	57 9	51 5	6 6	63 0	37 4
8	♂	45	14 4	1 8	9 6	15 2	50 8	48 9	49 9	36 0	30 5	31 4	12 0	57 4	27 1
Mean value			16 24	11 34	7 16	15 93	49 50	55 39	56 18	51 56	47 45	31 26	8 39	55 20	41 39
S E M			1 15	0 5	0 81	1 49	2 38	3 23	3 69	5 47	6 31	6 12	1 46	6 03	8 94

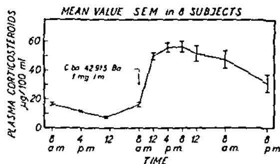


Fig 1 Plasma corticosteroids in eight non-endocrine patients (mean value ± S E M) on the day before the day of and the day after i m injection of 1 mg Ciba 42 915 Ba

RESULTS

Table I reproduces the values of plasma corticosteroids and urinary 17 KGS on the day before the day of and the day after injection of Ciba 42 915 Ba Mean values and standard error of the mean are given in the table and in Figs 1 and 2

The statistical significance of the corticosteroidogenic effect of Ciba 42 915 Ba has been evaluated by Student s *t* test A normal diurnal variation in plasma corticosteroids is found on the day before injection Mean plasma corticosteroids at 8 a m on the day before and at 8 a m immediately before injection are the same ($P > 0 1$) Four hours after injection mean plasma corticosteroids have increased significantly ($P < 0 001$) Maximum mean values are found 8–12 hours after injection A slight though not significant ($P > 0 1$) fall in plasma corticosteroids is found 16–24 hours after injection Thirty six hours after injection mean plasma corticosteroids have fallen significantly ($0 02 < P < 0 05$) although the mean value is still higher

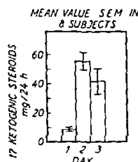


Fig 2 Urinary excretion of 17 ketogenic steroids in eight non-endocrine patients (mean value ± S E M) on the day before the day of and the day after i m injection of 1 mg Ciba 42 915 Ba

than the mean valu of plasma corticosteroids at 4 p m on the day b fore injection (0 01 < P < 0 02)

A significant increase in mean 24 hour 17 KGS on the day of injection is found (P < 0 001) A slight but not significant (P > 0 1) fall in 24 hour 17 KGS is found on the day after injection

No local reactions except slight tension and tendern ss were observed during and for some minutes aft r the injections

DISCUSSION

The present exp riment clearly shows that the modified synthetic beta[1] [4]corticotrophin (Ciba 42 915 Ba) has a prolonged corticosteroidogenic effect as reflected by the increase of plasma corti osteroids (cortisol + corticosterone) and uri nary 17 KGS This is in accordance with the re sults obtained by Gilboa et al (13) and Besser et al (5) In this study maximum levels in plasma corticosteroids are reached 8–12 hours after i m injection This has also been found by Besser et al (5) They found a significant clinical effect of 1 mg "depot tetracosactrin for about 32 hours while this study has demonstrated a significant clinical effect of the same dose for at least 36 hours

Besser et al (5) ob erved no significant dif ference between 80 units of corticotrophin gel and 1 and 2 mg "depot tetracosactrin" during the first four hours However the depot tetracosac trin maintained plasma corti osteroid levels long r than corticotrophin gel

From the results of Besser et al (5) and from the present study it may be concluded that to maintain continuously elevated plasma corticos teroids 1 mg of the depot preparation of *synthetic* beta[1] [4]corticotrophin need be given only once daily

Hypersensitivity reactions in man to ACTH of animal origin has b en described on several oc casions (7 12 14 29) However unmodified synthetic beta[1] [4]corticotrophin appears to have lower antigenicity In animal experiments only ex tremely small quantities of antibodies to beta[1] [4] corticotrophin have been demonstrated (8 10 11 17) It se ms evident that the immunogenic ac tivity of natural ACTH is chiefly attach d to the amino acids 25–39 (8 17) which explains the low antigenicity of beta[1] [4]corticotrophin

In clinical trials to date several patients hyp r s nsitive to natural ACTH preparations have been treated with beta[1] [4]corti o rophin without unto ward r actions (6 18 20 22 33)

The manufacture of d pot preparation of syn thetic beta[1] [4]corticotrophin has made prolonged treatment with beta[1–4]corticotrophin possible in man Experiments with this modified preparation are n ed d before any firm conclusions can be drawn concerning the antigenicity of b ta[1] [4]cor ticotrophin in man

REFERENCES

1 Asfeldt V H & Nielsen E Ugeskr Læg 1 7 855 1965
2 Bangham D R Mussett M V & Stack Dunne M P Bull Wld Hlth Org 27 395 1962
3 Barthe P., Desaulles P A Schar B & Staehelin, M Nature (Lond) 20 908 1964
4 Bell P H J Amer chem Soc 76 5565 1954
5 Besser G M Butler P W P & Plumpton, F S Brit med J 4 391 1967
6 El Shaboury A H Lan et 1 98 1965
7 Feinberg, S M Feinberg A R & Bi g, E J.A.M.A 147 40 1951
8 Felber J P Ashcroft S H Villanueva, A & Van notti, A Nature (Lond) 11 654 1966
9 Few J D J Endocr 2 31 1961
10 Fischer K., Hachmeister U & Kracht J Natur wissenschaften 52 347 1965
11 Fleischer N Givens, J R., Abe K., Nicholson W E & Liddle G W J clin. Invest 44 1047 1965
12 Forssman O Korsgren, M Nordh, B & Paulsen, F Acta allerg (Kbh) 18 462 1963
13 Gilboa Y Ber A & Winkelsberg, G J clin. Endocr 26 666 1966
14 Hill B H R & Swinburn P D Lancet 2 1218 19 4
15 Hofmann K., Yajima H Liu T Yanaihara N Chizuko Yanaihara & Humes J L J Amer chem Soc 84 4481 196
16 Hofmann K., Yajima, H Yanaihara, N Liu T & Lande S J Amer chem Soc 83 487 1961
17 Imura, H Sparks L L Grodsky G M & Forsham P H J clin Endocr 25 1361 1965
18 Jenny P M Muller A F & Mach, R S Schweiz. med Wschr 93 766 1963
19 Kappeler H & Schwyzer R Helv chim Acta 44 1136 1961
20 Karl H J Klin. Wschr 41 633 1963
21 Lamberg B A Strandstrom L & Pesonen S Acta med. scand 179 551 1966
22 Landon J James, V H T Cryer R J Wynn V & Frankland A W J clin Endocr 4 1 06 1964
23 Lee T H Lerner Aa B & Buettner Janusch V J Amer chem Soc. 81 6084 1959
24 Li, C H Vitam and Horm. 19 313 1961
25 Li, C H Meienhofer J Schnabel E Chung D Lo T & Ramachandran, J J Amer chem Soc 82 5760 1960

26 Moncloa F Velezco I & Gutierrez L A J clin Endocr 26 482 1966
27 Moor P de & Steeno O J Endocr 28 59 1963
28 Nielsen E & Asfeldt V H Scand J clin Lab Invest 20 185 1967
29 Rajka G Acta allerg (Kbh) 16 159 1961
30 Schuler W von Schar B & Desaulles P Schweiz med Wschr 93 1027 1963
31 Schwyzer R & Sieber P Nature (Lond) 199 172 1963
32 Shepherd R G Willson S D Howard K S Bell P H Davies D S Davis S B Eigner E A & Shakespeare N E J Amer chem Soc 78 5067 1956
33 Wood J B Frankland A W James V H T & Landon J Lancet 1 243 1965

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MASTOCYTOSIS TREATED WITH L HYOSCYAMINE (EGAZIL[1])

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Abst act A case of a 38 year-old woman with a mastocytosis syndrome is presented The urinary excre tion of histamine methylhistamine and 1 methyl-4 imid azole acetic acid was considerably higher than normally Treatment with *l* hyoscyam ne sulphate abolished gastritis diarrhoea and pressure induced pain, and reduced dermo graphism and itching However there was no effect on the abnormal urinary histamine excretion.

The concept of urticaria pigmentosa (u p) as a purely dermatological disease has changed to cover the generalized manifestation—systemic mastocytosis (s m) (24) It has also been accepted that s m at least in adult patients carries a cer tain risk of over morbidity and possibly also some over mortality Until recently treatment has been rather unsuccessful with the exception of a few patients in whom a histidine decarboxylase in hibitor has been used (18)

This communication reports on the effects of *l* hyoscyamine on some symptoms in a patient suff ring from s m Studies on the histamine metabolism were also performed

CASE REPORT

The patient was a 38 year-old married housewife who was adm tted to hospital in 1966 because of abdominal pain flushing and weight loss On admission there were three ma n groups of symptoms

1 *Gastrointest nal symptoms*

S nce at least 1940 she had had epigastric cramping pain heartburn and belch ng with acid taste appearing several times a year mostly in spring and autumn. These symptoms which appeared daily since the spring of 1965 were aggravated by hunger and also after meals, particularly when she ate pears, rosehip soup marzipan or acetyl salicylic ac d In 1953 a prepyloric ulcer was verified by X ray

Since youth and more since 1961 she usually had d arrhoea once after d nner In February 1966 after an upper respiratory infection she had transient diarrhoea 5–6 times a day with voluminous, yellow white faeces with glittering surface floating on water Since May 1965 she had unvoluntarily lost ten kg of weight

2 *Cutaneous symptoms*

The same agents that provoked abdominal discomfort also initiated a cutaneous flush with a feeling of heat mostly on chest and back, sometimes accompanied by itching and often by fatigue malaise and anxiety Ever since childhood she always reacted more than usual to scratches contact with stingin nettles, and to insect bites, with swelling and redness She had had a brownish exanthema since 1958

3 *Pain symptoms*

For several years the patient had experienced a deep smarting pain after pressure (e g blood pressure deter mination and embraces) which was most disa reeable Because of this feeling as of a thousand needles she could not lie still for any length of time especially not on a hard surface

Several doctors had ascribed her symptoms to nervousness and secondary flushin and abdominal pain in spite of the fact that she had always been careful to point out that the symptoms started with epigastralgia, then flushing appeared and then she became apprehensive

There were no laboratory findings indicating hyper thyro dism Intradermal testing with some 40 allergens including her own home dust, was negative

In March 1966 she was admitted to hospital for further in estigation of the abdominal symptoms.

There were few abnormal physical signs There was no enlargement of lymph nodes li er or spleen However over her body and extremities there were numerous millimetre sized, pigmented well delineated sl ghtly in filtrated maculopapules These appeared more clearly after blunt scratching In addition there was pronounced dermographism

Dermatologist's repo t

The clinical d agnosis was urti aria p gmentosa. Microscopically there was a dense infiltration of mast cells in the upper corium.

not expect the patient to develop such a strong flush and greatly increased epigastric pains during the first day of the treatment These symptoms reappeared when the patient resumed the treat ment after having neglected the tablets for a few days This transient deterioration could have been due to drug induced histamine liberation The histamine studies did not show a further increase in urinary excretion of histamine or its metabolites when Egazil treatment was again started For some reason however the patient did not develop the usual initial flush on this particular occasion

The mode of action of *l* hyoscyamine in producing the beneficial effects on several symptoms in the present case remains obscure The possibility of a spontaneous remission seems remote as such remission rarely occur in adults

The fact that the treatment relieved the gastric symptoms in this patient may or may not be connected with the systemic mastocytosis It could be regarded as the expected effect of an atropine like drug in any patient suffering from duodenal ulcer

It might be that the *l* hyoscyamine produced mast cell degranulation and histamine release This could explain the temporary aggravation of symptoms when treatment was started or was resumed after an interval The continuous administration of the drug could conceivably produce a continuous liberation of histamine which would keep the stores partially emptied and thereby prevent massive intermittent release However the measurements of histamine and metabolites in the urine do not provide support for such an action of the drug

Another tentative explanation might be lowered sensitivity to histamine The *l* hyoscyamine could have acted as an anti histaminic agent or it could in some indirect way have facilitated the adaptation of the organism to the increased amounts of histamine produced in this patient

As yet we have observed no untoward effects of the treatment Theoretically one might speculate that if a major a tion of atropine i mast cell degranulation this could produce osteosclerosis by lib ration of mucopolysaccharides and stimulation of ground substance formation (1) It seems less likely that such degranulation would cause osteoporosis by liberation of heparin which has been shown to induce bone resorption (16)

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REFERENCES

1 Asboe Hansen G Dermatologic aspects of mast cell activity Dermatologica (Basel) 1 8 51 1964
2 Braunsteiner H Das Mastocytose Syndrom Dtsch med Wschr 89 573 1964
3 Brogren N Duner H Hamrin B Pernow B Theander G & Waldenstrom J Urticaria pigmentosa (mastocytosis) Acta med scand 163 223 1959
4 Caplan R M Urticaria pigmentosa and systemic mastocytosis J Amer med Ass 194 1077 1965
5 Chieco-Bianchi L & Albano O Contributo alla conoscenza delle mastocitosi sistemiche (Segnalazione di un caso personale) Riv Anat pat Suppl 5 795 1963
6 Degos R Urticaire pigmentaire et mastocytoses Rev Prat (Paris) 13 601 1963
7 Demis D J & Brown D D Histidine metabolism in urticaria pigmentosa J invest Derm 36 253 1961
8 Demis D J Walton M D & Higdon R S Histaminuria in urticaria pigmentosa A M A Arch Derm 83 181 1961
9 Demis D J Walton M D Wooley D Wilner N & McNeil G Further studies of histidine and histamine metabolism in urticaria pigmentosa J invest Derm 37 513 1961
10 Demis D J The mastocytosis syndrome Clinical and biological studies Ann intern Med 59 194 1963
11 Demis D J & Zimmer J G Histaminuria in mastocytosis Arch intern Med 111 309 1963
12 Diamond J & Gross L Urticaria pigmentosa complicated by polycythemia vera Report of a case Blood 27 253 1966
13 Dotevall G & Walan A The effect of *l* hyoscyamine in tablets with sustained release on gastric secretion of acid in man Acta med scand 178 359 1965
14 Douthwaite A H & Hunt J N Effect of Nacton in patients with duodenal ulcer Brit med J 1 1030 1958
15 Granerus G & Magnusson R A method for semi quantitative determination of 1 methyl-4 imidazole-acetic acid in human urine Scand J clin Lab Invest 17 483 1965
16 Griffith G C Nichols G Asher J D & Flanagan B Heparin osteoporosis J Amer med Ass 193 91 1965
17 Jarnum S & Zacharиae H Mastocytosis (urticaria pigmentosa) of skin stomach and gut with malabsorption Gut 8 64 1967
18 Levine R J Histamine synthesis in man Inhibition by 4-bromo-3 hydroxybenzyloxyamine Science 154 1017 1966

19 Lindell S E Rorsman H & Westling H Histamine formation in urticaria pigmentosa Acta derm venerol (Stockh) 41 277 1961
20 Nettleship E & Tay W Rare forms of urticaria Brit med J 2 323 1869
21 Remy D Gewebsmastzellen und Mastzellen Reticulose (Funktionelle Zytologie und Klinik) Ergebn inn. Med Kinderheilk 17 132 1962
22 Sagher F & Even Paz Z The mast cells and mastocytosis With special reference to bone changes S Afr med J 35 470 1961
23 — Mastocytosis and the mast cell Karger Basel 1967
24 Selye H The mast cells Butterworths Washington 1965
25 Sollman T Manual of pharmacology Saunders London and Philadelphia 1948
26 Sun D C & Shay H Optimal effective dose of anticholinergic drug in peptic ulcer therapy Arch intern Med 97 442 1956
27 Wetterqvist H & White T Bioassay of histamine in human urine An improved method for purification of samples To be published
28 White T Histamine and methylhistamine in cat brain and other tissues Brit J Pharmacol 26 494 1966

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COMPARISON OF RUBELLA HAEMAGGLUTINATION INHIBITING AND NEUTRALIZING ANTIBODY CURVES IN NATURAL INFECTION

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Abstract Considerable differences are shown in rubella haemagglutination inhibiting and neutralizing antibody curves A rapid development of the haemagglutination inhibiting antibody to maximal titres is found within the first months of illness while the neutralizing antibody reaches maximum as late as 6 to 15 months after infection Both types of antibody persist at least 3 / years With respect to serodiagnosis of rubella the importance of obtaining the first serum sample as soon as possible after the appearance of rash is emphasized regardless of the type of antibody sought

In a preceding publication the development and persistence of neutralizing antibody in human rubella infection were reported (5) The study was based on a series of nine rubella patients bled at intervals from onset of illness over periods from 1 to 3 1/ years Since the haemagglutination inhibition (HI) techniques rec ntly described (3 7) app ar to offer certain advantages over the neutralization (NT) test in the serodiagnosis of rubella it was found desirable to have HI tests performed on the same series of sera in order to establish the HI antibody respons curve and to compare this with the previous findings pertaining to rubella NT antibody

MATERIAL AND METHODS

In previous publications detailed descriptions have been g ven of the human sera employed (5) and of the assay for rubella NT antibody (4)

Human sera were obtained from nine patients with a clinical diagnosis of rubella with rash Specimens were collected at intervals o er a period of 1? to 15 months with an additional blood sample from six pat ents 3 / years after infection All sera employed were heat inactivated at 56 C for 30 min and stored at minus 20 C until tested

NT tests were performed in rabbit cornea (SIRC) cells employing serial twofold dilutions of the sera starting at 1 4 All sera were tested with the addition of normal guinea pig serum to the medium used for dilution of sera and virus (4)

HI tests The haemagglutinating rubella antigen used in this study was supplied by Flow Laboratories Inc Rockville Md USA The sera were examined in the Takatzy microtitration system employing essentially the technique described by Halonen et al (3) All sera were absorbed with kaolin to remove non spe ific inhibitors and 0 9 saline made from deminerali ed water was used as diluent for serum and antigen Antibody titrations were performed employing twofold serial dilutions starting at 1 10

NT antibody titres have been calculated by the method of Karber and expressed as the reciprocal of the initial serum dilution in the serum virus inoculum The HI antibody t tres have been expressed as the reciprocal of the highest initial dilution of serum producing complete inhibition of haemagglutination Throughout this paper NT titres less than 4 and HI titres less than 10 are recorded as 0

RESULTS

The results are illustrated in Fig 1 and summarized in Table I For each patient two s ts of antibody titres have been recorded one set representing the HI antibody and the other set the NT antibody

HI antibody response curves

From Fig 1 it will be seen that rubella HI antibody in all patients appears rapidly after the onset of rash (day 0) rising sharply to maximal titres ranging from 160 to 1280 from 8 to 31 days later

In three patients (A C and F) there is a gradual decrease in titre from the maximal value while the HI antibody titre in the rest of the sera remains at the maximal level for periods varying up to 5 months forming a plateau on the HI antibody curve before the decrease in titre b gins In all patients rather low titres of 40 to 160 are seen 6 to 12 months after illness and the HI antibody persists at this level throughout the observation period of up to 3 1/ years

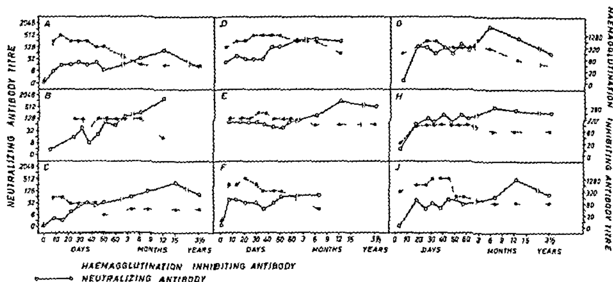


Fig 1 Haemagglutination inhibiting and neutralizing antibody curves for rubella patients A to J Day 0 = onset of rash

NT antibody response curves

The rubella neutralizing antibody curves shown in Fig 1 have been described in detail elsewhere (5) An either gradual or sharp rise in antibody titres is observed within a few days after rash increasing to maximal titres of 90 to 1450 as late as 6 to 15 months after infection A decrease in titre is seen later although NT antibody still persists 3 ½ years after illness

Comparison of HI and NT antibody titres

Table I rubella HI and NT antibody are compared with respect to the time when antibody is first demonstrated and to the time of appearance of maximal titres

It will be seen that HI antibody is demonstrable earlier than NT antibody in sera F G and J while the two forms of antibody can be demonstrated simultaneously in the rest of the patients A marked rise in the HI antibody titre is found after the first few days of illness reaching levels only two to eightfold lower than the maximum In one patient (C) the maximal HI titre is found as early as 8 days after rash and in all patients peak HI titres are reached within the first month of illness In contrast the first demonstrable NT

Table I *Comparison of rubella haemagglutination inhibiting (HI) and neutralizing (NT) antibody titres in individual sera A–J*

	Antibody first demonstrated				Maximal titre				Titre 3½ years after illness	
	Day		Titre		Day		Titre			
Pat	HI	NT	HI	NT	HI	NT	HI	NT	HI	NT
A	7	7	640	8	14	376	1280	90	40	11
B	6	6	80	4	25	378	320	1450		
C	8	8	160	6	8	457	160	360	40	90
D	4	4	120	23	27	182	1280	360		
E	7	7	320	90	31	367	640	10?4	160	51
F	1	7	10	45	21	02	1.80	90		
G	5	18	160	1 8	25	161	640	1450	40	64
H	4	4	0	4	17	193	160	51?	80	360
J	4	18	3?0	45	31	370	1 80	51	80	90

[a] Patient followed for 20? days only

antibody titres are low in one patient (B) several hundred fold lower than the maximal NT titre and the highest NT titres are not found until 6 to 15 months after illness Common to the two forms of antibody are relatively low titres in the 3 1/ year specimens

DISCUSSION

The serological diagnosis of virus infections is normally accomplished by examination of paired serum specimens collected from the patient 2 to 3 weeks apart early in illness

In a previous report (5) we stressed the necessity of collecting the first s rum sample in rubella infection at the app arance of rash or immediately thereafter in order to be able to demonstrate a fourfold or greater rise in neutralizing (NT) anti body titre The findings of the present study sug gest that prompt collection of the acute phase s rum sample is even more important when the s rological rubella diagnosis is based on the haemagglutination inhibition (HI) test since the HI antibody app ars earlier or at least simultane ously with the NT antibody Thus in three of the nine patients studied HI antibody was found in acute phase sera in which NT antibody was not yet demonstrable If serum samples collected dur ing the very first days after rash had been available from all patients the number of sera demonstrat ing HI antibody in the abs nce of NT antibody might have been even greater

If HI antibody is already demonstrable in th first blood specimen collected from a rubella suspect patient the possibility of a serodiagnosis based on the HI test will almost entirely depend on a prop r spacing of the paired serum samples Occasionally however the steep initial ris in HI antibody titres such as seen in four patients of this study may permit the demonstration of a significant titre rise even when the paired sera are collected with only a short interval If the first serum sample is not collected promptly at the onset of rash the present findings show that the generally employed interval of two weeks between blood samples is not always sufficient for a sig nificant HI antibody titre rise to d velop Further it will be seen that after the first app arance of HI antibody a fourfold titre rise may in most cases only be demonstrable if blood samples from the maximal titre period can be included in the test

The average time of app arance of maximal titres for the patients examined was day 22 with individual variations from day 8 to day 31 This finding suggests that in order to demonstrate a rise in HI antibody titre it may be necessary to collect blood specimens at weekly intervals until four we ks after the onset of rash It should be pointed out however that even if this is done a sero diagnosis based on HI antibody determinations may not always be feasible Thus it has not been possible to show a fourfold or greater rise in HI titre in the sera collected from patient E and the same would have applied to patients A and C if an early antibody negative serum sample had not been available

Rub lla HI titres have b en reported to be con siderably higher than the corresponding NT anti body titres (2 6 7) From the data in Fig 1 it is seen that HI as well at NT antibody titres of in dividual sera are highly dependent on the time of collection Thus if a comparison is made of the HI and NT antibody titres when they are first demonstrable Table I shows that eight of the nine patients studied have higher HI than NT titres while a comparison of the maximal titres reveals that only four of the nine patients show higher HI than NT antibodies In the s rum samples collected 3 1/ years after illness only one of the six pa tients from whom sera are available shows an HI antibody titre which is higher than the correspond ing NT titre The difference in the course of the HI and the NT antibody response curves ap parently reflects the d monstration of different antibodies rather than a higher sensitivity of one te t or the other

It is generally accepted that the protective value of s rum products is dep ndent on the contents of neutralizing antibody Our findings are therefore of considerable practical importance in relation to serum products intended for rubella prophylaxis These should not be selected on the basis of a high rubella HI titre since in many instances this is not correlated to a high NT antibody titre On the basis of the present study it is suggested that con valescent s rum" for passive rubella immunization should be derived from blood donors 6 to 15 months after clinical illness when th neutralizing antibodies have reached their maximum and not as is now common routine 3 to 6 weeks after onset of rash

Since in the present study HI and NT antibody

could be demonstrated simultaneously in all sera collected after the first few days of illness and because—as stated in a recent review by Dudgeon (1)—it appears that even very low levels of neutralizing antibody are indicative of immunity to rubella it seems permissible to use the HI test to determine the status of naturally acquired immunity to rubella

REFFRENCES

1 Dudgeon J A Maternal rubella and its effect on the foetus *Arch Dis Childh* 42 110 1967
2 Field A M Vandervelde E M Thompson K M & Hutchinson D N A comparison of the haemagglutination inhibition test and the neutralization test for the detection of rubella antibody Lancet 2 18 1967
3 Halonen P E Ryan J M & Stewart J A Rubella hemagglutinin prepared with alkaline extraction of virus grown in suspension culture of BHK 1 cells Proc Soc exp Biol 125 167 1967
4 Leerhøy J Rubella virus neutralization in heated sera Acta path. microbiol scand 73 475 1968
5 — Development and persistence of neutralizing antibody in human rubella infection *Acta* path microbiol scand In print
6 Lennette E H, Schmidt N J & Magoffin R L The hemagglutination inhibition test for rubella A comparison of its sensitivity to that of neutralization complement fixation and fluorescent antibody tests for diagnosis of infection and determination of immunity status *J Immunol* 99 785 1967
7 Steward G L Parkman P D Hopps H E Douglas R D Hamilton J P & Meyer H M Jr Rubella virus hemagglutination inhibition test New Engl J Med 276 554 1967

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HYDROXOCOBALAMIN

Excretion and Retention of Repeated Large Doses in Patients with Pernicious Anaemia

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Abstract The retention of hydroxocobalamin was found to be approximately the same after each of four 48 hourly injections of 1 mg of hydroxocobalamin in untreated pernicious anaemia After four such injections a maximum of 2–3 mg of the vitamin is retained corresponding to approximately 50 % of the normal stores

The retention of vitamin B_{12} after one injection is much higher for hydroxocobalamin (OH B_{12}) than for cyanocobalamin (CN B_{12}) OH B_{12} is thus more effective for replenishing B_{12} stores in a state of deficiency The normal amount of stored vitamin B_{12} has been calculated to be about 4 mg (3) After an injection of 1 mg which is the dose most commonly used 50–70 % is retained (1 2 4 6) This means that the restitution of the stores if completely empty will take some 8–10 injections Nothing is known however about the interval necessary between the injections It might be surmised that too frequent injections could result in progressively less retention due to saturation of the primary binding sites of the vitamin The present study was undertaken to find out the retention after four 48 hourly injections of 1 mg of OH B_{12} This regimen was chosen for practical reasons since most patients with untreated pernicious anaemia do not need hospital care for more than 8 to 10 days

MATERIAL AND METHODS

Sixteen patients with untreated pernicious anaemia were given by injection 1 mg of hydroxocobalamin every second day for eight days i e a total of 4 mg 24-hour urines were collected the volume measured and frozen aliquots stored until assayed Great care was taken not

Table I *24 hour urinary excretion of vitamin B_{12} following injections of 1 mg OH B_{12} on days 1 3 5 and 7*

Case no	Day 1		3	4	5	6	7	8	Total excretion (μg)	Calculated retention (mg)
1	47	3	121	16	166	44	233	76	706	3 3
2	322	4	318	13		17	251	25		
3	191	23	304	116	242	155	263	34	1330	2 6
4	281	34	215	60	285	112	328	60	1375	2 6
5	*208*	*242*	*346*	*76*	*21*	*137*	*272*	*68*	*1570*	2 9
6	416	86	459	137	312	75	235	57	1777	2 2
7	163	18	110	52	156	49	201	52	801	3 2
8	689	82	182	48	161	46	242	51	1501	2 5
9	171	12	218	30	267	78	289	63	1128	2 8
10	186	36	175	76	202	118	66	102	961	3 0
11	87	19	121	59	128	56	138	58	666	3 3
12	70	35	200	53	98	50	156	64	726	3
13	60	18	208	31	233	54	146	77	1027	3 0
14	14	14	160	30	188	56	02	65	857	3 1
15	157	12	157	16	238	53	308	63	1004	3 0
16	285	50	325	92	419	114	461	150	1896	I

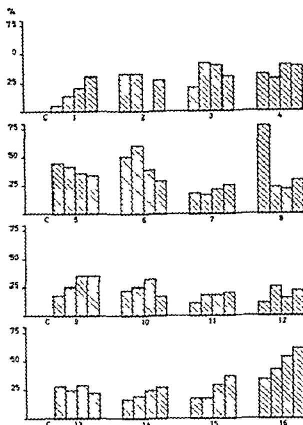


Fig 1 48 hourly urinary excretion of B_{12} after injection of 1 mg OH B four times on alternate days to 16 patients

to leave the urine samples at room temperature The vitamin B_{12} activity was estimated microbiologically according to Hutner et al (5)

RESULTS

All urinary excretion values for vitamin B_{12} are presented in Table I together with the calculated retention values These were found to be in the range of 2 1–3 3 mg with a mean value of 2 8 mg Th 48 hour urinary excretion results (i e the time between injections) are shown in Fig 1

The fifth to sixth day urine from case 2 was incompletely collected and is thus not included in the table or the figure As seen in Fig 1 three cases 1 15 and 16 and perhaps also case 9 show a continuous increase in urinary excretion In the other ten cases there is no su h obvious tendency suggesting a rather equal retention after each injection

The first 48 hour urinary excretion of case 8 was mu h higher (77%) than any of the other excretion values The reason for this is obscure

DISCUSSION

Whitey and Kilpatrick (8) compared the effect of repeated 48 hourly doses of 1 mg of CN B_{12} and OH B_{12} on the serum B_{12} levels in untreated cases of pernicious anaemia In one of their figures it can be seen that there was no significant difference between the urinary excretion after the first and *fourth injection in two patients* This agrees with the results that we have found and to our knowledge this is the only other observation published of a similar nature

The present study shows that the calculated retention of OH B_{12} after injections of 1 mg repeated every 48 hours is the same even after the fourth injection This kind of treatment will thus provide an amount of the vitamin approximately equalling 50–75% of an estimated normal B_{12} store of 4 mg Of course it must be borne in mind that these retention figures are probably somewhat too high There is a small excretion on the third and fourth day after an injection usually amounting to a few per cent or less Further the retention values are based on excretion figures which may be too low due to methodological shortcomings (7)

REFERENCES

1 Adams, J F & Kennedy E H J Lab clin Med 65 450 1965
2 Glass G B J Skeggs H R, Lee D H Jones, E L & Hardy W W Nature (Lond) 189 138 1961
3 Grasbeck R Nyberg, W & Reizenstein, P Proc Soc exp Biol (N Y) 97 780 1958
4 Hertz, H Kristensen H P O & Hoff Jørgensen E Scand J Haemat 1 5 1964
5 Hutner S H Bach M K & Ross G I M J Protozool 3 101 1956
6 Killander A & Schilling R F J Lab clin Med 57 553 1961
7 Killander A & Werner I to be published
8 Whitey J L & Kilpatrick G S Lancet 1 16 1964

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INTESTINAL CLOSTRIDIUM PERFRINGENS IN RHEUMATOID ARTHRITIS AND OTHER COLLAGEN DISEASES[1]

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Abstract The intestinal anaerobic flora has been stud ed w th special reference to Clostridium perfringens in various colla en diseases rheumatoid arthritis (RA) in particular and in a series of control cases The C perfringens flora was qual tatively and quantitatively abnormal in two thirds of 186 RA cases in two thirds of entero-arthritis cases in about half the SLE the psoriatic arthritis and the seronegative polyarthritis cases, but only in a few of the patients with pelvospondylitis ossificans and uro arthritis (Reiter s syndrome) An abnormal C perfringens flora was not demonstrated in any of the patient with polyarthritis or arthralgia after ton sillitis nor in any of the control cases The alpha anti toxin titre in serum was raised in 78 of the RA cases, and su h titre rises which occurred parallel to the pathologi al C perfringens flora were also demonstrated in the cases of other inflammatory rheumatic diseases but were not noted in any of the control cases

The results of faeces cultures and serologi al examina t ons are analysed in relation to several cl nical and laboratory parameters, such as the patient s age and sex, duration and activ ty of the disease administered drugs hypergammaglobul naemia and secretion of hydrochloric acid in the stomach A tendency to higher frequency of abnormal C perfringens flora is noted both in RA pa tients with high d sease activity and in elderly patients

In a previou paper (22) we reported briefly our observations relating to the intestinal Clostridium perfringens flora in rheumatoid arthritis and other collagen diseases and to the occurrence of both circulating and cell bound antibodies against C p rfringens alpha toxin in some of these diseases Here we present a more detailed report of our findings in an enlarged material and the methods used The relation of the findings to various clinical and laboratory parameters as well as their po sible pathogenetic significance will also be discussed

[1] Paper presented at the VIth European Rheumatology Congress O tober 1967 Lisbon

MATERIAL

The series comprises 381 patients with inflammatory rheumatic diseases who were treated at the Department of Rheumatology Karolinska sjukhuset or attended the hospital s outpatient department and 70 control cas s

Rheumatoid a thritis (RA)

186 patients All fit Ropes et al (30) criteria for a diagnosis of classical or definite RA The haemagglutina tion reaction with sensitized sheep-cells according to the technique of Svartz–Schlossman, was positive in 181 cases The remaining five patients had either shown a positive reaction earlier or undergone several courses of gold treatment, or had class cal erosive arthritis with ulnar deviation without signs of psoriasis SLE or ulcerat ve colitis

Se onegative polya th it s

45 patients forming a heterogeneous group including pa tients with probably incipient RA (with bilateral arthritis of small joints often developing in association with upper respiratory tra t infection) or polyarthritis involving mainly large joints in whom there was no ev dence of SLE arthropathia psoriatica pelvospondylit s urolo ical disease (Reiter s syndrome) or intestinal disorders of ulcerative-col tis or regional-enteritis type and in whom rheumatoid or antinuclear factors could not be demon strated

Arthr tis o arthralgia afte tonsill tis

18 patients All had histories of throat infection with findin s of group A streptococci n cultures of pharyngeal secretion and or raised antistreptolysin 0 titre n serum

Uro-a th itis

16 patients in whom the d agnosis was based on Olhagen s (26) cr ter a This group includes cases of post gonorrhoeal arthr tis and of Reiter s syndrome

Entero-a th itis

1? patients, whose jo nt trouble started in d rect associa tion with diarrhoeal d sease (enteritis or colitis)

Table I *Intestinal C perfringens flora*

		Qualitatively[a] and/or quantitatively[b] abnormal		Dubiously[c] abnormal		Normal	
Diagnosis	No of cases	No		No		No	%
Rheumatoid arthritis	186	125	67 1	15	8 1	46	24 8
Entero-arthritis	12	8	67	0	—	4	33
Systemic lupus erythematosus	27	16	59 3	4	14 8	7	5 9
Psoriatic arthropathy	23	12	52 2	4	17 4	7	30 4
Seronegative polyarthritis	45	21	46 7	5	11 1	19	42 2
Pelvo spondylitis ossificans	21	5	23 8	2	9 5	14	66 7
Uro-arthritis (Reiter s syndrome + postgonorrhoeal arthritis)	16	3	18 7	1	6 3	12	75 0
Arthritis or arthralgia after tonsillitis	18	0	—	5	27 8	13	72 8
Controls	70	0	—	7	10 0	63	90 0

[a] Qualitatively abnormal Nagler zones > 5 mm diam
[b] Quantitatively abnormal 10^5 bacteria per gram faeces
[c] Dubiously abnormal Nagler zones 4–5 diam and/or $> 10^3$ to $< 10^5$ bacteria per gram faeces

Pelvospondylitis ossificans

21 patients in whom X ray examinations showed findings consistent with bilateral sacroiliac joint arthritis and paravertebral syndesmophytes according to Romanus et Yden s (9) radiographic criteria

Arthropathia psoriatica

23 patients who had typical psoriatic lesions together with erosive arthritis of characteristic localisation and in whom tests for rheumatoid factor were negative

Systemic lupus erythematosus (S L E)

27 patients with arthritis or arthralgia or a history of these disorders and evidence of present or previous visceral manifestations raised ESR and in most cases hypergammaglobulinaemia more than a few L E cells and or high titres of antinuclear factors

Control series

46 healthy volunteers belonging to the hospital personnel and 24 arthrosis patients age range 18–79 years

METHODS

A *Examination of Faeces*

General comment

Patients who had been treated with sulpha drugs or antibiotics less than two weeks before the sampling were not included As most of the patients had an active disease some had taken antirheumatic drugs during the sampling period mostly moderate amounts of acetyl salicylic acid 1–3 g daily Some of the R A patients had received prednisolone about 5 mg daily (or an equivalent dose of some other steroid) About one third of the patients had also had a chloroquine preparation 200–250 mg daily The figures shown in Table I refer throughout to the results of the first examination at admission to the hospital irrespectively of whether samples taken later were positive The outpatients brought with them faeces samples at their first visit to the rheumatological department

Bacteriological examination of faecal samples

5 g of a fresh faecal sample stored at +4 C for at most 4 hours were transferred to a flask Physiological saline solution was added to make a total volume of 50 ml and homogenisation was done by careful stirring If not used for culture immediately the material was kept at − 0 C

Comparative studies using a dilution fluid other than physiological saline such as a buffer solution peptone water etc have shown that the physiological saline solution has no inhibitory effect on the C perfringens flora Equally freezing to − 20 C has not decimated the C perfringens flora (but has had this effect on other groups of microbes such as coliform bacteria)

The number of C perfringens was determined by ten fold dilutions as follows To a series of tubes was added 4 5 ml of physiological saline 0 5 ml of the material to be examined was transferred to the first tube After change of pipette the content of this tube was mixed and 0 5 ml was then transferred to the next tube and so forth At the same time 1 ml of each dilution was transferred to a series of tubes containing 1% lactose broth and 1 ml to each of three series of Petri dishes. To the first of these was added 5% horse blood agar to the second the same medium, and to the third 5 egg yolk agar The two latter plate series were incubated anaerobically (hydrogen gas environment) and the others aerobically Cultures were made at 37 C for 10–1 hours. The number of C perfringens was determined by counting the colonies in egg yolk agar and blood agar The count was made in two successive plates in the series. The diameters of the precipitation zones in egg yolk agar indicating the lecithinase production were measured The

Table II *Serum levels of C perfringens alpha-antitoxin*

Diagnosis	No of cases	Titre (0–0 01 U/ml) No		Titre (>0 01–0 10 U/ml) No	
Rheumatoid arthrit s	160	31	21 9	125	78 1
Entero-arthritis	7	1		6	
Systemic lupus erythematosus	25	10	40 0	15	60 0
Psoriatic arthropathy	15	7	46 7	8	53 3
Seronegative polyarthritis	38	19	50 0	19	50 0
Pelvo spondylitis ossificans	20	13	65 0	7	35 0
Uro-arthritis	15	12	80 2	3	19 8
Arthrit s or arthralgia after tonsillitis	13	12	92 5	1	7 5
Controls	50	50	100	0	—

strains which were typed in ordinary way are to be designated as C perfringens A more detailed description of certain properties common to strains belonging to the different groups of the material studied will be given later on (?4)

B *Serologic Analysis*

Determination of the alpha-ant toxin titre in blood serum

Serum was inactivated at 50 C for 30 minutes Dried filtrate of strain B 607 C (C perfringens Wellcome Research Laboratories) which lacks a detectable amount of theta toxin was used as test toxin (alpha toxin) The dilution fluid was calcium gelatin saline (Cagsal) 138 75 ml of 1 CaCl solution 100 ml of 5 gelatin physiological saline 2[illegible]5 g of NaCl and distilled water to 2 5 l Six % fresh sheep-erythrocyte suspension was used as ind cator (the sheep cells were washed three times)

The serum samples were tested in small tubes (Dreyer s tubes) Equal amounts of serum were tested against various dilutions of the test toxin (T/100 T/ 00 T/300 T/400 T=2 units of alpha toxin per ml) The content was mixed and the tubes were allowed to stand at room temperature for 30 minutes The same volume of sheep-cell suspension (as that of serum and test toxin) was then added After mixing the tubes were heated to 37 C in a water bath for 60 minutes kept at +4 overni ht and read

RESULTS

The results of the faeces cultivations are shown in Table I A *qualitatively abnormal flora* refers here only to the size of the precipitation zone in egg yolk agar (Nagler zone more than 5 mm in diameter) *doubtfully abnormal* refers to Nagler zones 4–5 mm in diam ter A *quantitatively abnormal flora* refers to 10 C perfringens bacteria per g of faeces or more *Doubtfully abnormal* means $> 10^3$ and < 10 bacteria per g of faeces It will be seen from Table I that up to now we have not found Nagler zones larger than 5 mm in the control cases that two-thirds of the R A patients had a definitely abnormal flora and that the few entero arthritis patients had the same high frequency of a pathological C perfringens flora Next in order are the S L E psoriatic arthritis and seronegative polyarthritis patients of whom 59 % 52 % and 47 respectively showed an abnormal flora On the other hand among the post infectious joint reactions there is no patient with an abnormal flora in the group of *arthritis after tonsillitis In the uro-arthritis* group there are three patients with an abnormal flora including one with chronic Reiter s disease and recurrent diarrhoeas Of the five pelvospondylitis patients with an abnormal C perfringens flora three had intestinal disorders manifested by diarrhoea

The result of the serological examination set out in Table II shows a marked parallelism with the result of the faeces cultures insofar as a pathological C perfringens flora is accompanied by raised *alpha antitoxin titre In rheumatoid* arthritis the frequency of pathological serum reactions is slightly higher than that of an abnormal C perfringens flora

This discrepancy may be due to several causes One of these is that the diet or occasional drugs might have inhibited the growth temporarily another cause is that in patients with low counts and high lecithinase activity the demonstration of a few bacteria will be due to chance Longitudinal studies have shown seemingly inexplicable fluctuations in the faecal C *perfringens flora,* which have also been observed by earlier investigators in studies of intestinal clostridia in other situations However we have not found

Table III *Frequency of abnormal C perfringens flora in relation to patient s sex in the rheumatoid arthritis group*

	No of patients		
	Total	Men	Women
	186	61	124
Abnormal flora	67 1	70 5	66 2

any correlation between abnormal C perfringens flora and altered intestinal motility represented by frequent stools or constipation

Relations of the findings to diverse variables

The result of the faecal cultivations and serum examinations were analysed with respect to a number of variables of possible significance The large R A group was used as the test object in these analyses The sex distribution shows the expected preponderance for women (Table III) The frequency of an abnormal C perfringens flora was about the same in both sexes however A comparison between the frequency of positive findings in the faeces and raised serum antitoxin titres respectively shows a rising tendency for higher ages as far as the occurrence of an abnormal faecal flora is concerned (Table IV) In the control series there are 16 arthrosis patients in the age group 50–79 years none of whom had an abnormal C perfringens flora One explanation of the higher frequency of an abnormal C perfringens flora with increasing age might be that a longer duration of disease can be expected in these categories An analysis of the frequency of an abnormal C perfringens flora and raised antitoxin titres respectively in relation to the duration of illness (Table V) shows however no overrepresentation for a longer period of illness But the number of patients with a short disease course (less than 1 year) is too small to allow any conclusions as regards this part of the material

Table V *Frequency of abnormal C perfringens flora and of raised alpha-antitoxin titre in serum in relation to duration of the disease (rheumatoid arthritis)*

	Abnormal flora		Raised alpha antitoxin titre (>0 01-0 1 U/ml)	
Duration	No of cases	( )	No of cases	( )
3 mo	/3		2/3	
3–12 mo	10/14	7.	8/1.	
1–5 y	?5/4?	59	27/36	75
5–10 y	3?/48	67	31/38	8
10 y	56/79	71	57/71	80
Total	1.5/186	67	1 5/160	8

The frequency of an abnormal C perfringens flora in relation to the ESR as indicator of the disease activity (6) shows a tendency to increase with increasing ESR as will be seen from Table VI 93 % of the R A patients with an ESR of 100 mm or more in one hour had an abnormal C perfringens flora The serum antitoxin response shows no such correlation however The series includes a few patients with low ESR and virtually inactive disease in whom all the same an abnormal C perfringens flora could be demonstrated

The rheumatoid arthritis series was analysed for the possible effect of some drugs on the C

Table IV *Frequency of abnormal C perfringens flora and of raised alpha-antitoxin titre in serum in relation to patient s age (rheumatoid arthritis)*

No of investigated cases	186		160	
	Abnormal flora		Raised alpha antitoxin titre	
Age group (y)	No of cases	of the group	No of cases	of the group
10–19	1 4		? 3	
20–.9	3 10		4/6	
30–39	7/14	50	9/14	64
40–49	3 36	64	.6 31	84
50–59	37 5	70	41 46	89
60–69	40 5?	77	9/41	71
70–79	14/18	78	14/18	78

perfringens flora (Table VII) No essential differences were noted for aspirin or small doses of prednisolone but it will be seen from the table that the R A patients with a normal flora were to a somewhat greater extent treated with chloroquine preparations This therapy may thus possibly have led to slightly too low figures for the number of patients with an abnormal flora However 33 R A patients with an abnormal flora had no drug treatment at the time of sampling

A measure of the progress of the disease is the occurrence of erosions in bones demonstrated by X ray Eighty of the R A patients had such erosions An abnormal C perfringens flora could be demonstrated in 70 7 of these patients as against 67 1° in the patients without erosive arthritis that is no significant difference The question whether or not the presence of achlorhydria would be of any significance to the C perfringens flora was studied by means of the Diagnex test in 100 inpatients with chronic arthritis (Table VIII) An abnormal C perfringens flora was noted in 62 7° of the Diagnex positive patients and in 61 % of those with a negative test or a weak reaction thus there was no difference

An analysis for the relation of an abnormal C perfringens flora to ordinary rheumaserological parameters such as rheumatoid factor anti nuclear factors antistreptolysin titres and anti staphylolysin titres provided no evidence of such a relationship Both an abnormal C perfringens flora and raised antitoxin titres occurred for instance in patients with negative rheumatoid factor test as in most of the S L E patients in all the psoriatic arthropathy and all the ent ro arthritis patients Nor was the antitoxin titre correlated with hypergammaglobulinaemia (here defined as a relative gammaglobulin concentration of more than 20 on paper-electrophoretic serum analysis) Of the R A patients 33 4 % had hypergammaglobulinaemia according to this definition 66° of these showed an abnormal C perfringens flora and 78 % had raised antitoxin titres as against 67 and 78 % respectively of the whole R A group thus no deviation from the R A group as a whole As regards S L E 22 out of 27 patients had hypergammaglobulinaemia 12 having raised antitoxin titres while three had such rises of titre and normal gammaglobulin values among them two nephrosis patients There was no exclusive correlation between the presence of joint symptoms and an abnormal C perfringens flora or raised antitoxin titres in S L E two patients with lupus nephritis alone had an abnormal C perfringens flora in faeces and raised antitoxin titre respectively

Table VI *Frequency of abnormal C perfringens flora and of raised alpha-antitoxin titre in relation to ESR as indicator of disease activity in the rheumatoid arthritis group*

	Abnormal flora		Raised alpha antitoxin titre	
ESR (mm/h)	No of cases	( )	No of cases	( )
< 15	13/22	59	16/19	84
15–50	47/77	61	50/64	78
51 99	51/72	71	47/61	77
≥ 100	14/15	93	12/16	75
Total	125/186	67	1*5/160	78

Table VII *Frequency of drug treatment in relation to abnormal and normal C perfringens flora in rheumatoid arthritis cases*

	Patient groups	
Type of drug treatment/d	Abnormal flora treated	Normal flora treated
Acetylsalicylic acid preparations (1–3 g)	65	57
Prednisolone (5–7 5 mg)	29	13
Chloroquine preparations (0 25 g)	30	41

Table VIII *Gastric secretion of acid studied by Diagnex test in relation to frequency of abnormal C perfringens flora in 100 consecutive cases of chronic arthritis*

	No of cases	Abnormal flora ( )
Diagnex test positive	51	62 7
Diagnex test negative or weak reaction	49	61 3

Tubeless gastric analysis by means of estimation of azuresin in the urine after ingestion of this dye compound (Diagnex Blue) and caffeine stimulation

DISCUSSION

Clostridium perfringens belongs to the normal intestinal flora and occurs mainly in the colon Sporadically it has also be n isolated in healthy

persons in samples aspirated through an intestinal tube both from the jejunum and the ileum (14) In comparison with the other anaerobes—lactobacilli bacteroides and fusiforms which are the predominating organisms in the intestine (8)—the clostridia form a minority (10) Probably because of technical difficulties of cultivation the part played by intestinal clostridia in the production of disease has attracted little interest in comparison with the studies of the aerobic intestinal bacteria The Clostridium flora has been studied mainly in connection with local disorders of intestinal function such as food poisoning diarrhoea in infants and fermentative dyspepsia However at the beginning of the 20th century quite a number of studies were made of the intestinal flora in pernicious anaemia in which it was found that invariably C perfringens occurred in greatly increased amounts in the intestinal contents (11) Of particular interest here is Kahn s (13) study in 1924 of the anaerobic flora in man with special reference to some conditions of then unknown aetiology notably pernicious anaemia but also obscure diarrhoeas and chronic eczema Kahn also investigated six cases of chronic arthritis All six had greater amounts of spore bearing anaerobes in the faeces than had the control cases All had C perfringens and three had also C sporogenes Kahn rejected the idea that these bacteria would be of pathogenetic significance no serological or immunological studies were made Månsson and Colldahl (21) studied the faecal clostridium flora in patients with bronchial asthma and found a significant increase of the number of C perfringens In *preliminary studies they also found an increase* of the C perfringens flora in rheumatoid arthritis no immunological studies were made

In the investigation presented here it has been shown that particularly in rheumatoid arthritis but also in several other inflammatory rheumatic diseases the intestinal C perfringens flora is significantly increased and that it has undergone a qualitative change The strains isolated by us are mostly characterized by high lecithinase activity (alpha toxin production) readily demonstrable by the Nagler effect in egg yolk medium but also by some other properties which seem to distinguish them from clostridia both in patients with achylia and in asthmatics namely their heat sensitivity and fermentative ability vis à vis inulin (24)

A new observation is the finding of an immunological response of the host organism manifested by circulating antibodies demonstrated with alpha toxin as test substance Cell bound antibodies have also been demonstrated by skin testing with a purified alpha toxin revealing in virtually all the R A patients a positive allergic reaction of delayed hypersensitivity type (22 27)

The reported observations give rise to the question whether the findings would have any pathogenetic implications in the collagen diseases That intestinal disorders can play a part in the causation of joint disease was noted as early as 1672 by Sydenham who pointed out that patients with dysentery can sometimes develop arthritis In the 19th century Ruhr rheumatismus (dysenteric rheumatism) was an established concept which assumed a new dimension when Reiter (28) and Fiessinger and Leroy (9) observed that in the late course of dysentery there sometimes appeared a triad of symptoms referable to the urinary tract eyes and joints So-called colitis arthritis appears in 10–20 % of patients with ulcerative colitis (39) and regional enteritis can also be complicated by arthritis involving mainly the sacroiliac joints and the spine The rare Whipple s disease illustrates that a clinically silent intestinal affection of probably bacterial origin (7) can give rise to joint symptoms several decades before the intestinal symptoms appear It has also been proposed that rheumatoid arthritis might be of enteric aetiology In the 1910s–20s a popular concept among some workers was that intestinal autointoxication originating from the upper parts of the colon was the cause of rheumatoid ar*thritis These ideas led to for instance drastic* sometimes apparently successful attempts at treating R A by colectomy (15 36) Courses of diet directed against putrefactive bacteria in the intestine were also recommended (4) Shatin (32, 33 34) advocates that R A is a disease of intestinal aetiology Excessive consumption of gluten containing food particularly rye and wheat products would according to Shatin lead to malabsorption with secondary amino-acid deficiency

The first specific evidence for the theory that intestinal clostridia can elicit a peripheral immunological reaction in the host was presented in veterinary medicine by Månsson (16 17 18 19 20) in his studies on parakeratosis in pigs. Månsson found that the development of para

keratosis was directly related to an increase of C perfringens in the intestine and to the influence of the antigens of these microbes on the host as evidenced by raised antitoxin titre in s rum elevated ESR and hyp rgammaglobulin aemia Mansson et al (23 25) in experiments with a high protein diet in pigs were able to show that the parakeratosis is preceded by arthritis involving the interphalangeal joints and accompani d by hypergammaglobulinaemia and raised antitoxin titre in serum

A fact of more than historical interest is that Achalme (1) in a fatal case of acute articular rheumatism" with carditis recovered from heart blood cardiac valves and pericardium anaerobic bacteria with the sam propertie as those later attributed to C perfringens by Welch and Fraenkel Bacillus d Achalme the name also given to this perfringens species at that time was isolated from both blood and joint fluid in acute poly arthritis by several investigators in the 1890s and the early 20th century (2 3 12 31 37 38) Up to World War I many workers believed that it was the cause of rheumatic fever Bosc and Carrieu (5) showed however that spores of B welchii can be isolated in great numbers from the skin of man and from these investigations they concluded that Bacillus d Achalme is a banal saprophyte which had contaminated the cultures from blood or other material Simonds (35) also questioned the validity of the concept that B d Achalme would be the cause of acute articular rheumatism because unlike the findings in gas gangrene autopsy of arthritis cases had not shown any foamy organs

We look upon the findings of an abnormal C perfringens flora as evidence of a change in the intestinal habitat We have started investigations with the object of finding out the origin of this disturbance partly by analysis of various conditions attended by functional changes in the glands of the digestive tract As was mentioned earlier the presence or absence of hydrochloric acid in gastric juice does not seem to play any decisive part Preliminary studies suggest that equally reduced salivary secretion as in chronic sialoadenitis does not lead to a change in the C perfringens flora Investigations into the part played by diet and the influence of antibiotics are also in progress

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REFERENCES

1 Achalme P Examen bactériologique d un cas de rhumatisme articulaire aigu mort de rhumatisme cerebral C R. Soc Biol (Paris) 43 651 1891
2 — Recherches bactériologiques sur le rhumatisme articulaire aigu Ann. de l Inst Pasteur 11 845 1897
3 — A propos du bacille du rhumatisme articulaire aigu. C R. Soc Biol (Paris) 75 82 1913
4 Bassler A The colon in connection with chronic arthritis J Amer med Sci. 180 351 19_0
5 Bosc F S & Carrieu M Le bacille d Achalme est un saprophyte banal hote habituel de la peau des rhumatisants et depourvu de toute spécificité pour le rhumatisme C R Soc Biol (Paris) 74 1229 1913
6 Bottiger L. E Malmqvist E & Olhagen, B Serum protein bound carbohydrates in rheumatic disease Ann rheum Dis. 23 495 1964
7 Caroli, F La malad e de Whipple Concours med 86 4677 1964
8 Dubos R., Schaedler R. W & Costello R Composition, alteration and effects of intestinal flora Fed Proc 22 132? 1963
9 Fiessin er N & Léroy E Contribution à l etude d une épidemie de dysentérie dans la Somme Bull Soc Med Paris, 40 _030 1916
10 Haenel H Spezielle medizinische Mikrobiolog e Zbl Bakt 176 305 1960
11 Herter C. A On bacterial processes in the intestinal tract in some cases of advanced anaemia with especial reference to infection with B aerogenes capsulatus J biol Chem 1 415 1906
12 Hewlett R. T Probable identity of Achalme s bacillus of acute rheumatism and the Bacillus enteritidis sporogenes of Klein Lancet 1 705 1901
13 Kahn, M C Intestinal spore bearing bacteria J infect Dis. 35 4?3 19 4
14 Kaiser M H Cohen R Arteaga, I Yawn E Mayoral L Hoffert W R. & Frazier D Normal *viral and bacterial flora of the human small and* large intestine New Engl J Med 274 558 1966
15 Mutch N Alimentary infections in chronic arthritis Lancet 2 1 66 19?1
16 Månsson, I The intestinal flora with special reference to atypical Clostridium perfringens and clinical observations Acta vet scand 5 _79 1964
17 — Electrophoretic stud es of blood serum the erythrocyte sedimentation rate and haemoglobin determinations. Acta vet. scand 5 287 1964
18 — Serological studies of blood serum and some skin tests Acta vet scand 5 295 1964
19 — Determination of zinc levels in blood and urine Acta vet scand 5 305 1964
0 Månsson, I & Olsson, B Quantitati e studies of coliforms, enterococci and clostridia in the faeces of pigs self fed a high protein and high-calcium diet. Acta agricult scand 11 197 1961

21 Månsson I & Colldahl H The intestinal flora in patients with bronchial asthma and rheumatoid arthritis. Acta allerg (Kbh) 20 94 1965
22 Månsson, I & Olhagen B. Intestinal Clostridium perfringens in rheumatoid arthritis and other connective tissue disorders Acta rheum scand 12 167 1966
23 — Intestinal Clostridium perfringens in arthritis and parakeratosis induced by dietary factors Experimental studies in pigs Bull Off int Epiz. 67 1319 1967
24 — To be published
25 Månsson I Olhagen B & Bjorklund N E Intestinal Clostridium perfringens in arthritis and parakeratosis induced by dietary factors Experimental studies in pigs (Abstract) VI Europ Congr Rheum Lisbon October 1967
26 Olhagen B Chronic uro-polyarthritis in the male Acta med scand 163 339 1960
27 Olhagen B & Månsson I Cutaneous sensitivity to Clostridium perfringens alpha toxin in rheumatoid arthritis and other collagen diseases To be published
28 Reiter H Über eine bisher unerkannte Spirochaeten infektion Dtsch med Wschr 42 1535 1916
29 Romanus R. & Ydén S Pelvospondylitis ossificans (rheumatoid or ankylosing spondylitis) A roentgenological and clinical guide to its early diagnosis Munksgaard Copenhagen 1955
30 Ropes M W Bennet G A Cobb S Jacox R & Jessar R A Diagnostic criteria for rheumatoid arthritis Ann rheum Dis. 18 49 1959
31 Rosenthal G Les rapports des variétés banales et rhumatismales du bacille d Achalme — bacille anaérobie du rhumatisme articulaire aigu et bacille perfringens — démontrés par l action identique croisée de sérum T R. La culture virus fixe du bacille perfringens C R Soc Biol 66 1027 1909
32 Shatin, R Concept of intestinal etiology in the pathogenesis of syndromes Acta rheum scand 10 246 1964
33 — The epidemiology of rheumatoid arthritis and human ecology Acta rheum scand 11 161 1965
34 — Gluten the small intestine and rheumatoid arthritis Rheumatism 2 48 1966
35 *Simonds J P Studies in Bacillus Welchii* Rockefeller Inst Med Pos Monographs 5 1915
36 Smith R The surgical relief of intestinal foci of infection in cases of arthritis deformans. Ann Surg 76 515 19 2
37 Thiroloix J Examen bactériologique du sang de deux malades atteints de rhumatisme articulaire aigu C R. Soc Biol (Paris) 49 .68 1897
38 — Bactériologie du rhumatisme articulaire aigu C R Soc. Biol (Paris) 49 945 1897
39 Wright V & Watkinson, G Th arthritis of ulcerative colitis Brit med J . 670 1965

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BENCE JONES PROTEINURIA IN BENIGN MONOCLONAL GAMMAPATHIES

Incidence and Characteristics

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Abstract The possibility and the extent to which detection of Bence Jones proteinuria might be used as a clinical differential criterion between benign and malignant monoclonal gammapathies has been investigated Clinical and laboratory studies were carried out in 42 subjects with an essential" serum M-component and by comparison in 46 patients with myelomatosis and in ten with macroglobulinemia Waldenstrom

The heat precipitation test was found weakly positive in only one urine sample from the benign cases (2.3%) as compared with 32 6 of the myeloma cases and 20 of those with macroglobulinemia. However by combining agarose-electrophoresis and immunoelectrophoresis of concentrated urine a urinary light chain component was demonstrated in 23 8% of the benign gammapathies For myelomatosis and primary macroglobulinemia the corresponding figures were 67 3 and 60% respectively

The amount of Bence Jones proteinuria never exceeded 60 mg/l in the benign forms whereas in the malignant forms it changed considerably from case to case and could reach values of as much as 10 g/l

Wide variations of pH and temperature were studied for their effect upon the thermal behaviour of two kappa type Bence Jones proteins isolated from the urines of two subjects with benign monoclonal gammapathies Within the limits investigated the physico-chemical properties of these benign type Bence Jones proteins did not differ apparently from those described as characteristic of Bence Jones protein in myelomatosis

The kappa lambda ratio of the serum M-components in the essential cases did not show any preferential synthesis as compared with myelomatosis and normal immunoglobulins This indicates that the benign monoclonal mutation of kappa and lambda synthesizing cells may develop at a rate proportional to their normal occurrence

Now that the electrophoretic and immunoelectrophoretic techniques have become routine procedures in many laboratories detection of serum and/or urine pathological proteins or M-components is no longer a rare occurrence

Initially considered as pathognomonic of myelomatosis and macroglobulinemia Waldenstrom M-components have been subsequently found in a large group of patients with different diseases (2 8 9 18 25 27 28 30 39) and in apparently healthy subjects as well (1 10 15) The condition of an M-component outside plasma cell myeloma and related malignancies has been termed "essential benign monoclonal gammapathy by one of the present authors (J W) (34–37) and occurs much more frequently than the corresponding malignant forms (1 16)

Recently the prognostic and diagnostic problems connected with the benign monoclonal gammapathies have been extensively reviewed (16) Among the differential criteria against myelomatosis (usually low remarkably constant levels of the serum M component absence of skeletal involvement and of diffuse plasmacytosis follow up studies showing status quo etc) the absence of Bence Jones proteinuria at least to any marked degree has been emphasized However the possible excretion of such microglobulins in amounts slightly exceeding the normal level has not been sufficiently elucidated in these forms and it still remains unsolved as to whether and to what extent the demonstration of Bence Jones proteinuria is compatible with a diagnosis of benign monoclonal gammapathy

In the present study clinical and laboratory investigations were undertaken to ascertain the incidence of Bence Jones proteinuria in a group of benign forms as compared with another group

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Table I *Number sex and age of the cases studied*

No of cases	Sex ♂	Sex ♀	Age Mean	Age Range
Benign monoclonal gammapathies				
4	19	23	66 2	32-91
Myelomatosis				
46	25	21	65 5	47 94
Macroglobulinemia Waldenstrom				
10	6	4	6? 4	49 8

of overt myelomas and macroglobulinemias The light chain typing of M components in both group is also reported

MATERIAL AND METHODS

Benign monoclonal gammapathies

Forty two subjects belonging to this group were examined All of them had been included in Hallén s thesis (16) and satisfied the s lection criteria listed in that paper The follow up period ranged from two years and 11 months to 13 years, and all subjects had been after-examined in the spring of 1965

Malignant forms

Forty six patients had myelomatosis and ten had primary macroglobulinemia Diagnoses were established on the basis of the common clinical radiological and cytological criteria The patients had been followed up by one of the authors (J W) and were last after-examined at the time of serum and urine collection They were almost all on Alkeran® treatment

In Table I sex and age (mean and range) of both groups are given

With the exception of the two cases of IgD-myelomatosis of which only frozen sera and urines were available in the remaining cases fresh serum samples and urines were obtained in the period Oct 1967 – Feb 1968

Protein characterization

Serum was separated in two tubes and stored at −20 C 24 hour urine output was filtered through a double thickness of Whatman no 1 filter paper in a cool room, and 100 ml samples were allowed to concentrate about 200-fold in the cold Collodium shells (Membranfiltergesellschaft Gottingen, Germany) connected to a vacuum pump were used throughout

Electrophoresis was carried out on 205 × 110 × 1 mm glass plates covered with 1% agarose in barbital buffer pH 8 6 0 75 M Ten different samples could be separated at one time on each plate and serum and concentrated urine from the same patient were always run in parallel to allow inspective comparison After the staining with Amido Black 10 B the patterns were scanned with a Vitatron automatic densitometer

Whenever the Heller test (stratification of urine over concentrated nitric acid) was positive the urinary protein was precipitated by 20% trichloroacetic acid Total protein determinations in both sera and redissolved urinary precipitates were done by the biuret method

If the electrophoretic pattern of the concentrated urine showed only a discrete component the amount of Bence Jones proteinuria was arbitrarily assumed to be equal to the total urinary protein content When as was commonly the case other protein fractions were clearly recognizable the electropherogram of the concentrated urine was scanned and the excretion of Bence Jones protein was then calculated as percentag of the total protein content

Immunoelectrophoresis was performed by the micromodification of Scheidegger (29) as described elsewhere (7) Total horse antihuman antiserum and strictly specific anti IgG anti IgA and anti IgM rabbit antisera were obtained from the Netherlands Red Cross Blood Transfusion Service Amsterdam Anti kappa and anti lambda rabbit antisera were prepared in this laboratory by intramuscular injection of kappa or lambda chains respectively isolated from the urines of two patients with myelomatosis The schedule of immunization and the procedures of absorption were as described by Fahey and McLaughlin (12)

Kappa and lambda antigenic determinants of the M components in sera and when present of free light chains in concentrated urines were studied by immunoelectrophoresis For IgM-components which did not penetrate the agar the sera were tested after dilution incubation with 0 1 *M* cysteine or increasing the ionic strength of the buffer In some cases isolation of the M-component was achieved by gel filtration on Sephadex G 200 of 1 ml serum eluted by phosphate buffered saline

The heat precipitation test for Bence Jones protein was performed in all urine samples in the manner described by Putnam et al (26) The urines were always acidified by the addition of 2 *M* acetate buffer pH 4 9 and the tubes were then incubated in a water bath (Ultra Thermostat Lauda Messgerate Lauda West Germany) at 56 C for 30 min Afterwards the tubes which showed any precipitation or opacity were heated at boiling temperature for 3 min in the same apparatus and inspected for possible decrease of the precipitate or clearing of the opacity Temperature and pH precipitation curves (26) were recorded for two purified urinary Bence Jones proteins isolated from two cases of benign monoclonal gammapathies

Isolation of Bence Jones proteins (for the preparation of antisera and the study of their physico-chemical characteristics) was carried out by 70% ammonium sulphate saturation of individual 24-hour urine samples After stirring for six hours at room temperature the mixture was allowed to stand overnight at +4 C The precipitate recovered by centrifugation, was washed thrice with a 70 solution of ammonium sulphate and then suspended in a small amount of sodium bicarbonate 0 1 *M* The salts were finally removed by exhaustive dialysis against running tap water Further purification of Bence Jones proteins was obtained by anionic chromatography on DEAE Sephadex A 50 using a gradient elution with 0 1 to 0 30 *M* phosphate buffer pH 8

Table V *Light chain types of serum M components*

Total kappa/lambda ratio	GammaG K	GammaG L	GammaG K/L	GammaA K	GammaA L	GammaA K/L	L-chain (Gamma μ) K	L-chain (Gamma μ) L	L-chain (Gamma μ) K/L	GammaD K	GammaD L	GammaD K/L	GammaM K	GammaM L	GammaM K/L
Benign monoclonal gammapathies															
1 4	23	11	2 0	2	6	0 3	—	—	—	—	—	—	—	—	—
Myelomatosis															
1 0	17	7	2 4	4	11	0 3	3	2	1 5	0	2	—	—	—	—
Macroglobulinemia Waldenstrom															
2 3	—	—	—	—	—	—	—	—	—	—	—	—	7	3	2 3

n cious anemia was diagnosed and appropriate treatment started Since then R.B C have always been about 4 mill

At routine examination in 1960 she appeared in a fairly good general condition ESR 58 mm/h, M-component 2 4 g/100 ml and gamma fraction 0 7 g/100 ml Bone marrow plasma cells were 5° and X ray was negati e

In 1961 she was treated for bronchopneumonia Plasma cells and gamma fraction were unvaried the M-component was ?2 g/100 ml.

The findings were mostly unchanged in 1964 (M-component 2 4 g/100 ml gamma fraction 0 8 g/100 ml plasma cells 9) at the after-examination in the spring of 1965 (M-component ?6 g/100 ml gamma fraction 0 8 g/100 ml plasma cells 3%) and in 1967 (M-component 2.5 g/100 ml gamma fraction 0 7 g/100 ml)

At the last after-examination in Feb 1968 after the discovery of a urinary l ght chain component the serum M-component was 3 0 g/100 ml and the gamma fraction 0 7 g/100 ml plasma cells were 5% and skeletal X ray was ne ative Physical examination showed good general conditions

Light chain typing

Serum M components and when pres nt urinary B nce Jones proteins were studied in all cases for their kappa or lambda antigenic determinants The purpose of this investigation was to establish wheth r or not M-components of the benign type exhibit a preferential light chain type

In Table V the frequencies of kappa and lambda *antigenic determinants both in the benign and* malignant forms are given It can be seen that in the b nign monoclonal gammapathies the total kappa/lambda ratio does not differ very much from that found in myelomato is And notwith standing the rather small number of cases ex amin d the differences are not statistically sig nificant even when the kappa/lambda ratios cal culated for each immunolo ical type are compared

DISCUSSION

The occurrence of light chains in the urines from normal adults has long been known (13 32) These normal free light chains are not derived from the degradation of gamma globulin but re present anabolic product of gamma globulin metabolism (14 31 33) The level has been cal culated in 2 5 mg of l chains/l urine (3) and hence remarkable concentrations of large urine samples are necessary for detecting these normal light chains

The main purpose of the present investigation was to assess wh ther and to what extent detec tion of a Bence Jones proteinuria in amounts ex ceeding the normal level could be used as a dif ferential diagnostic criterion between b nign mono clonal gammapathies and myelomatosis or macro-globulinemia Indeed rather few and somewhat contrasting results are reported in the literature on this subject Hallen (16) found a light chain component in the urine in four out of 90 subjects with benign monoclonal gammapathies In one of them myelomatosis could not be excluded and in another a prostatic cancer with skeletal metastases was detected at necropsy There were no signs of myelomatosis Hobbs (18) found no case of benign M-component with Bence Jones proteinuria in excess of 1 mg/100 ml Danon et al (9) observed a positive heat precipitation test in two out of 66 such cases but in 30 of the subjects with low proteinuria urinary immunoglobulin abnor malities could be also demonstrated

In the present material fresh serum and urine samples were examined in all but two cases of IgD myelomatosis Moreover the same techniques were used throughout so as to ensure compar

Table IV Data on ten cases of benign monoclonal gammapathy with Bence Jones proteinuria at [illegible] examination (February 1968)

Identification no[a]	Sex	Age	Hb (g/100 ml)	R B C (mill)	Leucocytes	Thrombocytes	Bone marrow plasma cells	Skeletal X ray	Total serum protein (g/100 ml)	Serum M component: Concentration (g/100 ml)	Serum M component: Immunological type	Serum M component: Light chain type	Bence Jones proteinuria (mg/l)	Blood urea nitrogen (mg)	Serum creatinin (mg)	Observation time (y)	Diagnosis or state of health
5	[illegible]	79	11 9	3 7	4 8	[illegible]		[illegible]	7 8	3 0	IgG	Lambda	58 4	40	1 2	10 3	Cholecystitis
10	♀	77	N T[b]	N T[b]	N T[b]	N T[b]	[illegible]		7 0	1 7	IgG	Lambda	19 9	N T[b]	N T[b]	3 1	Sarcoidosis
11	♂	60	[illegible]	[illegible]	4 5	156	5	[illegible]	[illegible]	1 7	IgG	Kappa	22 4	38	1 2	11 10	Healthy blood donor
24	♂	58	[illegible]	3 7	8 0	214	2	Arthr hip		[illegible]	IgG	Kappa	27 3	42	1 4	3 0	Cerebral atrophy
29	♀	70	11 6	3 9	3 8	180	3	Normal	6 8		[illegible]	Lambda	17 0	34	1 3	8 4	Diabetes mellitus
35	[illegible]	68	11 1	3 6	4 6	180	3	Normal	[illegible]	0 8		[illegible]mbda	14 0	36	N T[b]	3 0	Hypothyroidism
45	♂	71	14 7	4 5	6 3	214	2	Normal	7 1	0 4	IgG	[illegible]	13 5	42	1 2	3 4	Healthy
49	♂	70	N T[b]	N T[b]	N T[b]	N T[b]	N T[b]	N T[b]	7 0	0 5	IgG	Kappa	[illegible]	N T[b]	N T[b]	3 9	Prostatic hyperplasia
56	♂	74	14 7	4 7	6 1	210	5	Normal	7 6	0 4	IgG	Kappa		48	1 7	10 5	Encephalomal sequelae
60	♀	79	14 6	4 2	4 5	188	2	Normal	6 7	0 4	IgG	Lambda	14 0		1 0	3 0	Healthy

[a] Identification numbers are the same as used by Hallén (16)   [b] N T = not tested

pended in acetate buffer at a concentration of 0 2 g% It was of kappa type in both cases Extensive variations of temperature and pH were studied in the heat precipitation test It was found that by keeping the protein concentration constant and the pH at 5 the precipitation started from 47 to 50 C and was maximal in the range 56 –60°C On the other hand pH precipitation curves made up in acetate buffers showed that for the same protein content and at 60 C the optimum precipitation occurred in the range of pH 4 9–5 3 The precipitation decreased slowly when approaching the alkaline side

Clinical studies

Although all the subjects included in this investigation had been selected on the basis of a reasonably sure diagnosis (16) the cases of benign monoclonal gammapathies in which Bence Jones proteinuria had been demonstrated were again submitted to a thourough after examination It included physical examination sternal puncture skeletal X ray (skull ribs thoracic and lumbar spine pelvis and proximal parts of the femora) determinations of hemoglobin red and white blood cells thrombocytes blood urea nitrogen and serum creatinine In two out of ten positive cases the after-examination was only partial because the patients could not or would not co-operate

A clinical and laboratory summary of the findings in these ten cases is shown in Table IV The data do not differ significantly from the corresponding values obtained for the same subjects at after-examination in the spring of 1965 (16)

The case history of patient no 5 (see Table IV) under clinical observation for over ten years will be reported in some detail because of a moderate increase of the serum M-component in the last year

Case 5

Female born in 1889 She had been largely healthy until Nov 1956 when she was admitted with symptoms of cholecystitis apparently without gallstones. RBC were *3 3 mill* and *FSR 1 0 mm/1 h* *Serum electrophoresis* disclosed an M-component (*IgG type 1.5 g/100 ml*) the concentration of the gamma fraction was 0 75 g 100 ml. X ray showed no osteolytic lesions.

In Oct 1958 she complained of tiredness, glossitis, weight loss RBC were 2 7 mill and W.B C 400 The M-component was 1 8 g/100 ml and the gamma fraction 0 7 g/100 ml Sternal puncture revealed 5 % plasma cells and some megaloblasts serum vitamin B was low Per

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Myelomatosis															
1 0	17	7	2 4	4	11	0 3	3	2	1 5	0	2	—	—	—	—
Macroglobulinemia Waldenstrom															
2 3	—	—	—	—	—	—	—	—	—	—	—	—	7	3	2 3

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In Table V the frequencies of kappa and lambda antigenic determinants both in the benign and malignant forms are given It can be seen that in the benign monoclonal gammapathies the total kappa/lambda ratio does not differ very much from that found in myelomatosis And notwithstanding the rather small number of cases examined the differences are not statistically significant even when the kappa/lambda ratios calculated for each immunological type are compared

DISCUSSION

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The main purpose of the present investigation was to assess whether and to what extent detection of a Bence Jones proteinuria in amounts exceeding the normal level could be used as a differential diagnostic criterion between benign monoclonal gammapathies and myelomatosis or macroglobulinemia Indeed rather few and somewhat contrasting results are reported in the literature on this subject Hallen (16) found a light chain component in the urine in four out of 90 subjects with benign monoclonal gammapathies In one of them myelomatosis could not be excluded and in another a prostatic cancer with skeletal metastases was detected at necropsy There were no signs of myelomatosis Hobbs (18) found no case of benign M component with Bence Jones proteinuria in excess of 1 mg/100 ml Danon et al (9) observed a positive heat precipitation test in two out of 66 such cases but in 30 of the subjects with low proteinuria urinary immunoglobulin abnormalities could be also demonstrated

In the present material fresh serum and urine samples were examined in all but two cases of IgD myelomatosis Moreover the same techniques were used throughout so as to ensure compar

able results It was found that the heat precipitation test is very seldom positive in the benign forms (one case out of 42) However by combining agarose electrophoresis and immunoelectrophoresis of concentrated urines Bence Jones proteinuria was unequivocally demonstrated in 23 8 of the benign monoclonal gammapathies Although the percentages of positivity were considerably higher in the malignant forms neither a positive thermoprecipitation test nor electrophoretic and/or immunoelectrophoretic abnormalities of concentrated urine could be demonstrated in about 33% of patients with myelomatosis and in 40% of those with primary macroglobulinemia

The calculated amounts of Bence Jones proteinuria never exceeded 60 mg/l in the benign forms whereas in myelomatosis the quantities varied remarkably from case to case and could be as high as 10 g/l Therefore the results suggest that Bence Jones proteinuria of more than 60 mg/l strongly argues for myelomatosis or macroglobulinemia Conversely the association of a serum M component in a rather low concentration and a urinary light chain component of less than 60 mg/l is not sufficient to warrant a diagnosis of malignancy

The occurrence of Bence Jones proteinuria outside myelomatosis and primary macroglobulinemia has been repeatedly reported in patients with malignant tumors and skeletal metastases (6 16 19 22) In our subjects with benign monoclonal gammapathies and a urinary light chain component thorough clinical and laboratory examinations were carried out after the discovery of the urinary abnormalities Nevertheless not only could myelomatosis or macroglobulinemia be excluded in all cases but also malignant tumours with skeletal metastases were not apparently found

The possibility that an individual case of essential paraproteinemia may represent myelomatosis or macroglobulinemia Waldenstrom in an early stage cannot be excluded and has actually been proved in isolated instances (5 20 24) But on the whole such a possibility seems to be of rare occurrence (16 35)

Whether or not all or most of the benign monoclonal gammapathies with a urinary light chain component will eventually develop myeloma is a problem which cannot be solved at the present time Although the observation time was in no case shorter than three years and ranged up to almost twelve years it is obvious that only further follow up will allow definite conclusions However it should be emphasized that with the only exception of case no 5 (Table IV) in which a moderate increase of the serum M-component was demonstrated in the other cases no clinical or laboratory findings suggested as compared with the after examination in the spring of 1965 (16) an evolution process towards malignancy

Wide variations of pH and temperature were tested for their effect upon the thermal behaviour of two kappa type Bence Jones proteins isolated from the urines of two subjects with benign monoclonal gammapathy The results suggested that within the limits investigated the physico-chemical properties of Bence Jones proteins from benign forms do not differ apparently from those described as characteristic of Bence Jones proteins in myelomatosis (26)

The frequency of kappa and lambda chains in a large number of serum M-components has been recently studied by Laurell and Snigurowicz (21) and by Wollheim and Snigurowicz (38) However no clinical distinction between benign and malignant forms was made by these authors On the other hand Hammack (17) claimed a preferential synthesis of kappa type light chains (20 cases out of 20) in a group of "idiopathic paraproteinemias Our present results in agreement with those of Danon et al (9) would seem to suggest that the total kappa/lambda ratio of serum M components in the benign forms does not differ significantly from the ratios found in myelomatosis and in normal immunoglobulins (4 11, 23) Therefore it seems reasonable to assume that the cells which synthesize kappa or lambda type immunoglobulins may undergo a benign monoclonal proliferation with a frequency roughly proportional to their normal occurrence

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REFERENCES

1 Axelsson, U Bachmann R & Hallén, J Frequency of pathological proteins (M-components) in 6995 sera from an adult population Acta med scand 179 235 1966
Bachmann R The diagnostic significance of the serum concentration of pathological proteins (M components) Acta med scand 178 801 1965

3 Berggård I & Edelman G M Normal counterparts to Ben e Jones proteins free L polypeptide chains of human gamma globulin Proc nat Acad Sci (Washington) 49 330 1963
4 Bernier G M & Cebra J J Frequency distribution of alpha gamma kappa and lambda polypeptide chains in human lymphoid tissues J Immunol 95 746 1965
5 Bloom M L Shulman S & Witebsky E Anticomplementary activity of multiple myeloma Clin Res 6 206 1958
6 Cr yssel R Les anomalies de la synthèse des gamma globulines Transfusion (Paris) 8 117 1965
7 Dammacco F & Clausen J Antibody deficiency in paraproteinemia Acta med scand 179 755 1966
8 Dammacco F & Bonomo L Paraproteinemia non mielomatosa p 57 Atti Giornate Pisane Malattie Immunologiche Disprotidemiche Pisa 1967
9 Danon F Clauvel J P & Seligmann M Les paraprotéines de type IgG et IgA en dehors de la malad e de Kahler Rev franç Étude clin Biol 12 681 1967
10 Derycke C, Fine J M & Boffa, G A Dysglobulinemies "essentielles" chez les sujets agés Nouv Rev franç Hemat 5 7_9 1965
11 Fahey J L Two types of 6 6 S globulins and 18 S macroglobulins in normal serum and gamma microglobulins in normal urine J Immunol 91 438 1963
12 Fahey J L & McLaughlin, C Preparation of antisera specific for 6 6 S gammaglobul ns beta_A globulins, gammal macroglobulins and for type I and II common gammaglobulin determinants J Immunol 91 484 1963
13 Franklin E C Physicochemical and immunologic studies of gamma globulins of normal human urine J clin Invest 38 159 1959
14 Gordon, D A Eisen A Z & Vaughan J H Studies on urinary gamma globulins in patients with rheumatoid arthritis Arthritis Rheum 9 575 1966
15 Hallén J Frequency of abnormal serum globul ns (M-components) in the aged Acta med scand 173 737 1963
16 — D screte gammaglobul n (M)components in serum Clinical study of 150 subjects without myelomatosis Acta med scand Suppl 46 1966
17 Hammack W J Idiopathic paraproteinemia Clin Res 13 274 1965
18 Hobbs J R Paraproteins benign or mal gnant Brit med J 3 699 1967
19 Hosley H F M proteins plasmacytosis and cancer Cancer _0 _95 1967
20 Kyle R A & Bayrd E D Benign" monoclonal gammapathy a potentially malignant cond tion Amer J Med 40 4 6 1966
21 Laurell C B & Sngurowicz J The frequency of kappa and lambda chains in pathologic serum gammaG gammaA gammaD and gammaμ immunoglobulins Scand J Haemat 4 46 1967
22 Magnus-Le v A Über die Myelomkrankhe t vom Stoffwechsel die Bence Jones-Proteinurie Ztschr Kl n Med 119 307 193_
23 Mannik, M & Kunkel H G Two major types of normal 7S gammaglobulin J exp Med 117 213 1963
24 Norgaard O Recherches sur l evolution preclinique du myelome multiple Acta med scand 176 137 1964
25 Osserman, E F & Takatsuki, K Plasma cell myeloma gamma globulin synthesis and structure A review of bioch mical and clinical data, with the description of a newly recognized and related syndrome $H_γ2$-chain (Franklin s) disease" Medicine (Baltimore) 4 357 1963
26 Putnam F W Easley C W Lynn, L T Ritchie A E & Phelps, R A The heat precipitation of Bence Jones proteins I Optimum conditions Arch biochem. Biophys 83 115 1959
27 Radl J & Masopust J Idiopathische Paraproteinamie Schweiz med Wschr 94 961 1964
28 Riva G Idiopathische und Begleitparaproteinamien Helv med Acta 31 285 1964
29 Scheidegger J J Une micro-méthode de l immunoelectrophorese Intern Arch Allergy 7 103 1955
30 Seligmann M Les globulines myelomateuses ne sont pas pathognomoniques de la maladie de Kahler Presse med 75 1631 1967
31 Solomon A Waldmann T A Fahey J L & McFarlane A S Metabolism of Bence Jones proteins J clin Invest 43 103 1964
32 Stevenson, G T Detection in normal urine of protein resemblin_ Bence Jones protein J cl n Invest 39 119_ 1960
33 — Further studies of the gamma related proteins of normal urine J clin Invest 41 1190 196
34 Waldenstrom J Abnormal proteins in myeloma Adv intern Med 5 398 1952
35 — Studies on conditions associated with disturbed gamma globulin formation (gammapathies) p 211 Harvey Lecture Academic Press New York 1961
36 — Hyper ammaglobulinemia as a clinical hematolo ical problem a study in the gammapath es Progr Hemat 3 266 196
37 — The occurrence of benign, essential monoclonal (M type) non macromolecular hypergammaglobulinemia and its differential diagnosis IV Studies in the gammapathies Acta med. scand 176 345 1964
38 Wollheim F A & Sngurowicz J Studies on the macroglobulins of human serum IV The frequency of light cha n types K and L in polyclonal and monoclonal gammaM Scand J Haemat 4 111 1967
39 Zawadzki, Z A & Edwards G A Dysimmunoglobulinemia in the absence of clinical features of multiple myeloma and macroglobulinemia Amer J Med 42 67 1967

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COMPLICATIONS IN MEASLES WITH SPECIAL REFERENCE TO ENCEPHALITIS

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Abstract In the 15 year period 1948–1962 4874 patients were hospitalised in the City and County of Copen hagen with measles All the case reports have been studied concerning compl cations and mortality rate spe ial attention being paid to the incidence and the prognosis of encephalitis The patients surviving from encephalitis have been followed up

The most se ere complications were as expected pneumonia and encephalitis Pneumonia was found in 1 69 patients 12 of these patients died Encephalitis was found in 68 cases among which six deaths Before the new therapeutic principles dating from the great polio epidemic in 1952 five of 21 patients died from ence phalitis, but after that time only one of 47 patients died Of the survivors from encephalitis 59 (95) were fol lowed up 3–16 years after the disease three suffered from grave defects 16 had mild defects and 40 had recovered completely

The frequency of encephalitis was 0 43‰ of the notified cases of measles in the period of investigation. Among the admitted patients 2 died (4.5‰) The mor tality rate in the notified cases was only 0 14‰

Encephalitis is a rare complication in measles and concerning mortality rate and sequelae is not so severe as considered previously

Vaccination against measles has been used on a large scale in many countries in the last 6–7 years In Denmark vaccination has been used only in some districts in Greenland (4) Before entering into a discussion whether to vaccinate the whole population or not it is of course desir able to know how severe the disease is and especially the incidence of encephalitis and the sequelae of this complication

In this paper complications other than ence phalitis will be only briefly mentioned where after encephalitis and its sequelae assessed after a follow up examination of th survivors will be discussed in detail

MATERIAL

In the 15 year period 1948–1962 4874 patients (616 males and 2258 females) with measles were admitted to hospitals in the City and County of Copenhagen, of whom 4730 to the Department for Contagious Diseases, Blegdamshospitalet and 144 developed measles during their stay in the pediatric departments in the City and County of Copenhagen (the pediatric departments of Rigshospitalet, Blegdamshospitalet Sundby Hospital and Amtssygehuset Gentofte and the Dronning Louise and Fuglebakken pediatric hospitals) In the region as a whole 157 300 cases of measles were notified during the same period As 298 700 children were born in this region in 1947–1961 the numbers of notified cases are not remarkably small.

The curves comparing the numbers of hospitalised cases with the numbers of notified cases in the eight epidemics in the period are s en to concur (Fig 1) Most of the hospitalised patients were in the a e-group 1–4 years (Table I) which does not mean that the disease is most common in this a e group as it is to be expected that the incidence of hospitalisation of patients with measles falls with increasing age In the age-group 4–5 months the disease does occur but relatively rarely In the age group 6–11 months measles is as common as in children above 12 months of a e as for comparison, a compensation must be made for the shorter time span of the younger group A relati ely large group were more than 20 years old this group includes 98 patients from Greenland who contracted measles during their stay in Copenhagen.

All the case reports were stud ed by the author her self

RESULTS

Complications

Complications were seen in 2470 (51) of the admitted 4874 patients with measles In 305 (6°) cases two or more complications occurred In 1585 (32) patients there were no complica tions In the remaining 17 the patients were

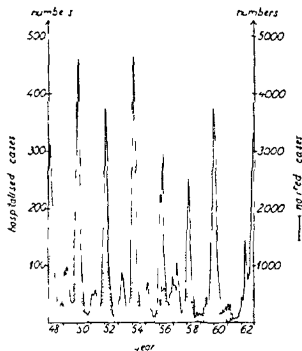


Fig 1 Measles Comparison between admitted and notified cases The numbers of cases are drawn from month to month In the eight epidemics during the period the two curves are seen to concur

admitted because of other diseases and measles appeared during their stay in the hospital The complications catarrhal otitis media acute bronchitis and acute enteritis will not be discussed

Pneumonia was a frequent complication being found in 1268 (26 0 %) cases (Fig 2) From the case reports it was impossible to say whether the cases were primary measles pneumonia or bacterial pneumonia The relative annual incidence was the same during the period of investigation (Fig 3) Twelve patients (0 9 %) died from pneumonia Bacteriological diagnoses were only made in five of these cases in two cases Staphylococcus aureus was found

Suppurative otitis media was found in 861 patients (17 7 %) but complicating osteitis only in three cases In two of the cases purulent meningitis developed without sequelae

Acute laryngitis was found as a complication in 213 patients (4 1 %) 154 males and 59 females whereas in no other complication was found a sex difference Ten of these patients were tracheostomised all of them were decannulated within ten days

Lymphocytic meningitis was found in 58 patients (1 2 %) In none of these cases did the case history or the course of the disease cause suspicion of encephalitis

Purulent meningitis was found in 13 cases (0 3 %) of these one case was fatal (meningococci) the other patients were discharged without sequelae

Encephalitis

Among the hospitalised patients encephalitis was found in 68 (1 4 %) 35 males and 33 females all but one between the ages of six months and 10 1/ years the average age being 5 years A single patient was 52 years old The annual number of cases within the period of investigation varied from zero to eleven (Fig 4)

The diagnosis encephalitis is in this publication based on the clinical picture In most of the cases encephalitis started with loss of or clouding of consciousness Convulsions alone were not regarded as a sign of encephalitis nor were transitory reflex changes in relation to convulsive fits without concurrent paralysis

The cerebral symptoms began from two to fourteen days (mean five days) after the onset of the rash and in 90 % with loss or clouding of consciousness (Fig 5) The spinal fluid was examined in 67 of the 68 cases *Pleocytosis* was found in 50 cases Quantitative determination of

Table I *Age incidence among 4874 patients admitted with measles*

Three of the patients were under four months of age none of their mothers had had measles Among 2 4 patients over 0 years of age 98 were Greenlanders who contracted measles *during their stay in Copenhagen*

Age (y)	No of cases	Per cent of total
1[a]	476	8 7[b]
1– 4	3203	65 9
5 9	854	17 5
10–14	89	1 8
15–19	78	1 6
> 0	224	4 6
Total	4874	

[a] 0–3 mo 3 patients 4–5 mo 34 patients 6–11 mo 387 patients
[b] The figure 8 7 in children under one year of age is only comparable with the percentages for the other groups when compensation has been made for the time span of the younger group

Sympt m (d mb)	d p f
Pneumonia (1268)	26 0
Otitis media supp (861)	17 7
Laryngitis ac (203)	4 2
Encephalitis ac. s c (68)	1 4
Meningitis lymf s c (58)	1 2
Meningitis purulent (13)	0 3

Fig Complications among 4874 patients admitted with measles. In 297 (6 1) two or more of the complications mentioned were found in 1585 (32.3%) no other disease than mea les. Besides these complications catarrhal otitis media, acute bronchitis and a^ute symptomati enteritis were found. Alto ether ^470 (^0 7^) of the admitted patients with measles had complications

protein in the spinal fluid was done in the cases admitted after 1951 Among 43 cases 20 patients had spinal protein above 40 mg per 100 ml Electroencephalography was performed only in 12 patients

Besides the symptoms mentioned in Fig 5 mutism was found in one case together with motor disturbances In another case the only symptoms were sudden deafness and motor disturbances

Follow up examination

In the acute stage of encephalitis six of 68 patients died all of the 62 primary survivors are known to be still alive The follow up examination of these patients comprises 59 (95) Forty five patients appeared for follow up examination while another 14 answered an enquiry by letter The observation period ranged from three to sixteen years being over five years for 50 (85°) of the examined patients

All the examinations were carried out by the author During the interview with the patients interest was concentrated on the patients intelligence how they had managed at school and whether behavioural changes had been noticed Attention was also focused on possible disturbances of gait and reflexes Neither spinal fluid examination nor ophthalmoscopy or ele^troencephalography were performed

From the results of the follow up examination it is seen (Table II) that 40 patients (68 %) had recovered completely while three suffered from grave defects Of these one had cerebral palsy and two were very feeble minded one of whom had already been suspected of being mentally retarded before the measles

From the table it is seen that six of the patients were mildly backward of whom two were known to have managed well at school before the encephalitis having severe difficulties at school after the disease As regards the other four patients it is not possible to say whether their backwardness resulted from the disease or whether they had been mentally retarded previously

Behavioural changes were met with in two patients Besides these two patients one patient

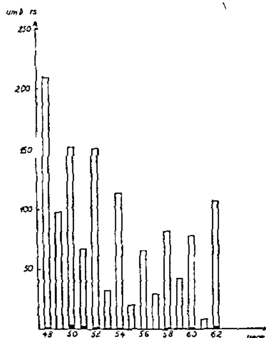


Fig 3 Pneumon a, total 1 68 The relati e annual number of cases was almost the same during the period of in estigation The black columns show the fatal cases

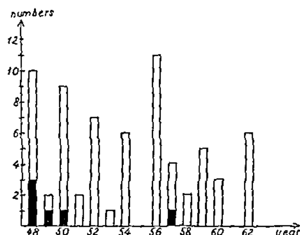


Fig 4 Encephalitis total 68 The annual number of cases varied from zero to eleven The black columns show the fatal cases

was found to have anxiety neurosis perhaps resulting from a knowledge of the encephalitis contracted 11 years previously

Epilepsy (petit mal) was found in one patient in whom the symptoms began four years after the encephalitis but this epilepsy well controlled no longer caused her any trouble as she had had no symptoms during eight years of treatment Grand mal was found in one of the two feeble minded patients

The patient who became deaf still had impaired hearing but managed well with a hearing aid

Headache was reported in two patients neither of whom used analgetics

The remaining three patients had mild and rather dubious sequelae One patient (now aged 18) still had enuresis 14 years after the disease before the encephalitis she had no troubles of this kind The remaining two patients (aged 14 and 16) had slight walking troubles neither reflex changes nor muscular weakness were found at the follow up examination

One boy had greatly impaired muscular strength in the legs and mutism but six months later he had recovered completely and was found normal at the follow up investigation three years later

As regards all patients there seems to have been proportionality between the period with clouding of consciousness and the liability to sequelae it seemed justifiable to exclude sequelae due to hypoxia

Fatal cases

Among the 68 patients with encephalitis six died their age being from six months to seven years These six deaths were distributed as follows during the first three years of the period of examination five deaths occurred among 21 patients in all while during the last twelve years only one of 47 patients died

Among the first five fatal cases two died of respiratory failure two during prolonged convulsive fits and one in hyperthermia during a complicating pneumonia The last of the six patients died from hemorrhage from the innominate artery after tracheostomy and intubation Autopsy was performed in all but one of the six cases Microscopic examination of the brain showed encephalitis

Of the admitted 4874 patients 22 died (4 5‰ of the admitted patients 0 14‰ of the notified 157 300 cases) Among the 1268 patients with pneumonia 12 (0 9°) died The frequency of death from pneumonia is nearly the same at the

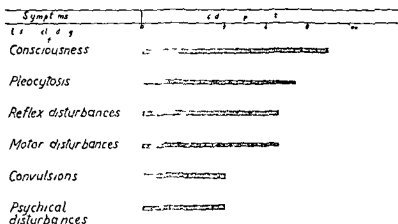


Fig 5 Incidence of symptoms in encephalitis Convulsions alone were not regarded as signs of encephalitis nor were transitory reflex changes in relation to convulsive fits without concurrent paralysis Quantitative determination of protein in spinal fluid was only performed in patients admitted after 1951 in these 43 patients spinal protein was above 40 mg in 0 cases

Table II *Follow up examination*

Among 59 (95) of the 62 survivors grave defects were found in three patients mild defects in 16 while 40 (68) had recovered completely from their encephalitis

Normal	40
Cerebral palsy	1
Feeble minded	2
Backwardness	6
Behavioural changes	2
Anxiety neurosis	1
Epilepsy (petit mal)	1
Surd tas	1
Headache	2
Enuresis nocturna	1
Walking troubles	2
Total	59

end of the period of investigation as at the beginning (Fig 3) The remaining four of the fatal cases included one patient with purulent meningitis (meningococcica) one with thrombocytopenia (before contracting measles this patient had megaloblastic anemia and cerebral palsy) one with appendicitis and one with miliary tuberculosis (besides measles this patient had whooping cough)

DISCUSSION

The period 1948–1962 was selected because it could be expected that the results would show that the prognosis had changed very much during this period In 1952 an unusually severe poliomyelitis epidemic raged in Denmark during which the principles of treatment of respiratory failure were fundamentally changed These new principles were naturally also applied if necessary to the treatment of measles The appearance of the many new antibiotics would probably have changed the prognosis in bacterial complications especially in pneumonia

The pneumonia mortality rate however is nearly the same throughout the whole period but may possibly decrease in the future as it is now feasible to make the bacteriological diagnosis so quickly that adequate therapy can be started within the first hour after admission to hospital The bacteriological diagnosis is made by direct microscopy of stained smears from the trachea obtained by suction through a nasal catheter

On the other hand the encephalitis mortality rate decreased remarkably during the period of investigation In the first three years five of 21 patients (24 %) died from this complication while in the last 12 years of the period only one patient (2 1 %) among 47 died This decreasing mortality rate is no doubt due to the improved treatment in accordance with the experience from the polio epidemic The innominate artery hemorrhage causing the only fatal case since 1951 is a known complication which has been seen twice among 623 cases of tracheostomy in Blegdams hospitalet (6)

The encephalitis mortality rate in the period of investigation was 9 % which is on a par with other investigations (2) In a Swedish publication (1) one death occurred among 64 cases of encephalitis in the period 1955–1964 This period may be compared with the period 1953–1962 in the present investigation in which one death was found among 38 patients The mortality rate is thus comparable with that of the Swedish publication

The frequency of encephalitis was 0 43‰ of the notified cases in the City and County of Copenhagen The incidence of encephalitis as a complication in measles is reported to range from 1‰ (1 3) to 2 5‰ (5) The variations may be caused by varying criteria for the diagnosis Some authors classify every symptom from the central nervous system as due to encephalitis which means that cases with secondary lymphocytic meningitis without other signs of central nervous disturbances than pleocytosis are included

Encephalitis is a rare complication in measles and concerning mortality rate and sequelae is not so severe as considered previously

REFERENCES

1 Holmgren B Kargsten S O Lindahl J & Sterner G Lakartidn 64 1088 1967
2 La Bocetta, A C & Tornay A S Amer J Dis Child 107 247 1964
3 Miller D L Brit med J 2 75 1964
4 Mordhorst C H Personal communication
5 Tyler H R Medicine 36 147 1957
6 Welin F Ugeskr Læg 126 1079 1964

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DISINFECTION OF THE HANDS OF WARD PERSONNEL

A Comparison of Six Disinfectants

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Abstract Six preparations for hand disinfection have been compared five hexachlorophene detergents and one quaternary ammonium compound In preliminary experiments one of the hexachlorophene preparations and the quaternary ammonium compound were excluded from further investigations because of heavy skin irritating properties and poor antibacterial effect The remaining four hexachlorophene preparations were compared using a Latin square design Three of the preparations gave a 93 5–97 5 per cent reduction of the resident hand flora the fourth disinfectant being significantly less effective One of the three preparations caused greater reduction in bacterial hand flora than the other disinfectants, which on the other hand have less side-effects Altogether the differences between the three superior preparations were small and the cost may therefore partly decide which is to be chosen During treatment with hexachlorophene preparations the frequency of Staph aureus isolations was greatly reduced but there seemed to be no influence on gram negative organisms

Disinfection of the hands is supposed to be one of the most important ways of controlling hospital infections (7 14 22) Various antiseptics have been found to reduce the bacterial flora of the hands markedly without causing serious skin irritation These should therefore replace the use of ordinary soap by hospital staff and probably also by patients (2 18) For such use hexachlorophene preparations seem to be the most suitable disinfectants at present

However within this group of antiseptics there are great variations in quality and cost It is well documented that the antibacterial effect of liquid preparations is superior to that of solid (8 10) but the effect of liquid preparations also seems to vary (10) Some of these disinfectants are said to inhibit not only the growth of gram positive organisms but also to some extent the growth of gram negative rods However the latter remains to be proved in practical tests Differences in side effects probably also exist (7)

Whilst dealing with prophylactic measures against surgical wound sepsis we have compared six hand washing preparations five containing hexachlorophene and one based on a quaternary ammonium compound

MATERIAL AND METHODS

The preparations numbered 1–6 (see below) were tested on 12 nurses and four laboratory technicians who during the treatment period used the preparations for all hand washing instead of soap The experimental design is described in relation to each experiment. The bacteriological methods however were the same in all experiments

The following antiseptic hand washing preparations were studied

1 Zalpon (Izal) a liquid soap containing 2 5% hexachlorophene
2 Nicasept (AB Tykefa) a detergent containing 2 % hexachlorophene
3 Sumasept (A/S Denofa og Lilleborg Fabriker) a detergent containing 3 hexachlorophene
4 Phisohex (Winthrop) a detergent containing 3% hexachlorophene
5 Steraskin (Pfizer) a detergent containing 3% hexachlorophene and 3 % chloroxylenol
6 Rodamin (Ferrosan) a detergent containing 1 2 benzalkonium chloride

The bacterial flora of the hands was investigated by a method described by Lowbury and Lilly (8) except for minor modifications The subjects washed their hands in 300 ml of sampling fluid in the following way both hands were moistened up to the wrist and then rubbed three times firmly to and fro first palm to palm then the right palm over the left dorsum the left palm over the right dorsum and last with the fingers interlacing followed by a final rinse This was repeated three times and the whole procedure lasted two min

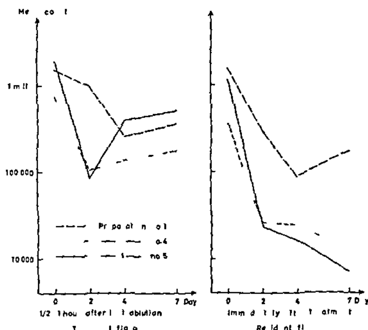


Fig 1 Mean counts of viable organisms before and during treatment with hexachlorophene detergents

The transient flora was investigated by a method described by Story (19) A truncated glass cylinder was placed on the sampling area Five ml of sampling fluid were measured into the cylinder and the skin was then systematically rubbed with a glass spreader for 30 sec

Dilutions of sampling fluid were inoculated on lactose agar blood agar and mannitol salt agar plates which were incubated at 37 C for ?4 40 and 48 h respectively The selection of pathogenic staphylococci was based on the capacity to coagulate human plasma

The sampling fluid was Ringer solution with 1 Tween 80" In inoculation tests this solution was found to neutralize the antibacterial effect of preparations 1 2 3 and 4 For preparations 5 and 6 tests for transfer of inhibitory concentrations of antiseptics to sampling fluid were made by inoculating control tubes and sampling fluid with dilutions of broth cultures of Staph aureus Staph albus and E coli Counts made from pour plates did not indicate transfer of any inhibitory amounts of antiseptics to the sampling fluid

EXPERIMENT I

Methods

Preparations 1 4 and 5 were tested on five subjects who used the disinfectants for all hand washing for one week each allowing one week of ordinary soap between each preparation for return of resident hand flora The preparations were us d in random sequence Sampling by hand washing was done ; -1 hour after the last ablution the subjects coming directly from duty Then the hands were washed with the preparation in a standard way under a running tap for one minute including a thorough rinsing and dried on a paper towel A second sample of hand washing was then obtained

Preparations 2 4 and 6 were tested on four subjects in similar experiments except that the first sampling was omitted and that ordinary soap was used for the ablution preceding the first day sampling

Results

The left side of Fig 1 shows the bacterial counts in hand washings from the subjects coming directly from duty representing mainly the transient flora (15) The right side of Fig 1 gives the mean counts in hand washings obtained immediately after the treatment representing the resident flora (9 15) Both the transient and the resident flora were reduced during treatment. The individual counts from the transient flora show great fluctuations most likely because of differences in external contamination between subjects and in the same individual at different times Thus by these experiments it seems difficult to distinguish the effect of the different preparations and these samplings were therefore omitted in further investigations

The results of the second sampling (resident flora) showed less fluctuations and suggested that preparation nos 4 and 5 were superior to no 1

Table I shows the results of the second part of the experiment. The reduction in the counts of viable organisms with preparation 6 was very small During treatment with no 2 two out of four subjects experienced considerable skin ir

Table I *Mean counts (in thousands) of viable organisms in hand washings before and during treatment with three antiseptic detergents*

Mean counts from four subjects

Preparation no	Before treatment	Day of treatment		
		2	4	7
2	758 4	140 2	102 0	174 8
4	778 9	35 2	15 2	25 1
6	515 9	106 8	328 7	350 0

irritation and as this detergent also caused only small bacterial reductions preparations 2 and 6 were excluded from further investigations

EXPERIMENT II

Transient Flora

Method

The residues of antiseptic on the skin after treatment with a preparation may kill some of the bacteria contaminating the skin This was investigated by a method similar to that described by Lowbury et al (11) A Latin square design was used to compare preparations 1 3 4 and 5 on four subjects each preparation being tested once on each individual The subjects first rinsed their hands for one minute in 70% ethyl alcohol to reduce the resident flora This was followed by two minutes treatment with the preparation under a running tap in a standard way including a final thorough rinsing The hands were dried on paper towels and immediately afterwards, 0 02 ml of a 0-hour broth culture of Staph aureus was inoculated on a marked area of the palm of the left hand and spread over the area with a glass rod. The film of Staph aureus was allowed to dry and left untouched for one hour before the area was sampled by the method described above The survival after washing with ordinary soap was elucidated in a separate experiment The numbers of infecting organisms were calculated from dilutions of the cultures used.

As the frequency distribution of bacterial skin counts approximates the log normal form the counts were transformed to a logarithmic scale

Results

Table II gives the counts of Staph aureus recovered The logarithms of the number of infecting organisms varied from 6 76 to 6 86 The counts of Staph aureus recovered by the sampling expressed as percentage of the number inoculated are given at the bottom of the table

By analysis of variance the counts after treatment with the disinfectants were found not to differ significantly However this limited experiment suggests that preparation no 1 is less effective than the others The superiority of disinfectants over ordinary soap is evident

Table II *Effect of residues of antiseptics on artificial contamination with Staph aureus*

Counts (log) of recovered organisms

Subject no	Preparation no				Ordinary soap
	1	3	4	5	
1	5 04	5 50	4 57	4 70	6 39
2	4 63	4 49	3 09	4 06	6 51
3	5 59	4 93	5 12	4 61	6 6
4	5 15	3 25	3 31	3 42	6 70
Mean log count	5 10	4 54	4 0[illegible]	4 20	6 43
Mean reduction (per cent of log count)	[illegible]5	33	41	38	6

EXPERIMENT III

Resident Flora

Method

In this experiment preparations 1 3 4 and 5 were compared on 12 subjects Each preparation was used for one week on each subject, allowing one week of ordinary soap between the treatment periods for return of the resident flora. A Latin square design was used in order to neutralize irrelevant factors The subjects were divided into three groups, each group being allotted to different Latin squares With only one week allowed for the return of the resident flora, the squares were arranged to neutralize 'carry-over' effect (3)

The resident flora was sampled after the standard hand washing with the detergent concerned the first sample for each preparation being obtained immediately before the subjects started the treatment Further samples were taken after [illegible], 4 and 7 days of use and 2 and 4 days after completing the treatment. As in the former experiments, the subjects were examined coming directly from duty [illegible] hour after the last ablution The counts were transformed to a logarithmic scale

Side-effects were recorded after interviewing and examinations at the end of the treatment with each preparation The side-effects were given scores according to the scale at the bottom of table VI

Results

Table III gives the counts (logarithms) of viable organisms in hand washings before treatment and the mean counts during treatment Subject no 2 had to interrupt the treatment with preparation no 1 because of heavy skin irritation after one day of use In the statistical analysis the missing value has been estimated as stated by Cox (3)

Table III *Logarithms of counts of viable organisms in hand washings before and during treatment with hexachlorophene detergents*

	Preparation no							
	1		3		4		5	
Subject no	B	D	B	D	B	D	B	D
1	6 03	5 18	5 54	4 53	5 93	4 26	5 34	3 84
2	5 73	4 19[a]	5 63	4 08	6 21	3 72	4 63	3 11
3	6 45	4 81	6 84	4 97	5 75	5 29	6 24	4 41
4	6 08	4 70	5 84	3 40	5 21	4 37	5 57	3 95
5	4 91	4 99	5 22	4 95	6 01	4 79	5 36	4 11
6	5 41	4 66	5 92	4 74	5 30	3 79	4 65	3 94
7	6 24	4 82	5 45	5 23	5 80	4 55	5 52	4 11
8	5 64	5 50	5 79	5 57	5 92	4 99	5 74	5 10
9	6 31	5 21	6 02	4 82	6 [illegible]3	4 94	5 79	3 38
10	4 73	3 53	4 82	3 48	5 08	2 91	4 74	2 92
11	5 42	4 81	5 38	3 87	5 96	4 22	6 08	4 10
12	5 52	5 05	5 75	4 [illegible]6	5 86	4 16	6 37	3 65
Mean log count	5 71	4 79	5 68	4 49	5 77	4 33	5 50	3 89
Mean count (not log)	899 200	175 700	1 009 800	92 800	776 200	65 400	641 000	27 700
Mean reduction	0 92		1 19		1 44		1 61	

B = before treatment D = during treatment
[a] Estimated value (see text)

Fig 2 shows the mean counts from all subj cts before during and after treatment with the dif ferent preparations The effect of the prepara tions is compared by analysing the differences be tween the pretreatment counts and the mean counts during treatment (Table III) Using the studentized range test the allowance for the mean log counts is $\pm 0\ 621$ ($P = 0\ 05$) Thus the difference between preparations 1 and 5 is sig nificant

By analysis of variance the mean counts before *treatment are not significantly different Accord* ingly the mean counts during treatment may be compared directly By the studentized range test the allowance is $\pm 0\ 408$ ($P = 0\ 05$) Accord ingly preparation 5 is significantly b tter than all the others and preparation 4 is significantly su perior to preparation 1 The differences betwe n preparations 3 and 4 and between 1 and 3 are not significant

The sum of the counts of Staph aureus in the hand washings from all subjects are shown in Table IV None of the subjects were permanent carriers and five never yielded Staph aureus in their hand washings during the experiment The results confirm that hexachlorophene treatment not only reduces the total bacterial counts in hand washings markedly but also reduces the counts of the more transient Staph aureus

Table V gives the sum of the counts of Gram negative bacilli in the hand washings One nurse was permanently carrying Gram negative organisms throughout the experiments The oth r isolations are most often from two or three cons cutive samples from the same subjects all but three sub jects giving samples with Gram negative bacilli on one or another occasion Evidently none of the preparations have any important effect on Gram *negative bacilli on the hands*

Table VI shows the side effects experienced The e were usually slight with preparations 3 4 and 5 By statistical analysis of the scores th allowance is $\pm 0\ 916$ ($P = 0\ 05$) Thus the side effects of preparations 3 and 4 are significantly less than the side effects of preparation 1 Pre paration 3 also has significantly less side effects than preparation 5 The differences b tween pre parations 3 and 4 between 4 and 5 and between 1 and 5 are not significant

DISCUSSION

Antiseptic preparations for hand washing have been studied by many authors (e g 4 6 8 10 11

12 13 16) Several methods have been employed but as the conditions of the experiments often differ considerably from the practical use by hospital ward personnel (10) the results are not applicable to hospital ward routine In this investigation however the experiments were especially d signed to fit practical ward routine

Hand-disinfecting agents should replace the use of ordinary soap by ward personnel and probably also by patients (2 18) The reduction of *resident* flora of the hands by regular use of hexachloro phene preparations is comparable with povidone iodine surgical scrub and alcoholic chlorhexidine (11 12) But as povidone iodine has all its effect at the time of application (12) and as alcoholic chlorhexidin or the chlorhexidine handlotion re commended by Rubbo and Gardner (17) is to b used after ordinary soap washing we find these preparations less suitable for ward personnel than the hexachlorophene preparations

The effect of hexachlorophene in liquid detergents on resident flora of the hands is well documented Our investigation documents significant differences between liquid preparations During use of hexachlorophene containing compounds hexachlorophene is deposited in the skin (5 20) and as the deposition in the skin depends on the vehicle of transmission (20) the differences between liquid preparations are easily explained

Of the six antiseptics tested preparations 3 4 and 5 are thought to give satisfactory results with a reduction in the resident hand flora of 93 5–97 5

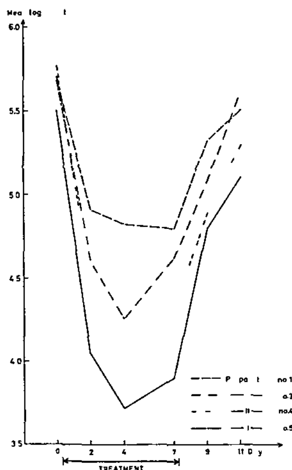


Fig 2 Mean counts (logarithms) of viable organisms in hand washings before during and after treatment with hexachlorophene detergents

Table IV *Total counts (in thousands) of Staph aureus in hand washings before and during treatment with hexachlorophene detergents*

Preparation no	Day before treatment 5	3	0	Day of treatment	4	7
1	3 5 (1)	119 0 (2)	151 5 (2)	3 0 (2)	18 0 (2)	0 0 (0)
3	6 5 (3)	11 5 (3)	319 0 (3)	·.0 (2)	0 0 (0)	14 5 (1)
4	34 0 (1)	3? 0 (3)	34 0 (3)	0 0 (0)	0 0 (0)	0 0 (0)
5	6 0 ( )	17 5 (1)	306 0 (3)	0 0 (0)	0 0 (0)	0 5 (1)
Total	?0 0 (7)	180 0 (9)	810 5 (11)	5 0 (4)	18 0 (2)	15 0 ( )

F gures in parentheses indicate the number of isolations of Staph aureus

Table V *Total counts (in thousands) of Gram negative bacilli in hand washings before and during treatment with hexachlorophene detergents*

	Day before treatment			Day of treatment		
Preparation no	5	3	0	2	4	7
1	37 0 (1)	199 0 (1)	54 5 (2)	101 0 (2)	131 5 (1)	6 0 (3)
3	219 5 (1)	223 0 (5)	60 5 (4)	95 5 (2)	167 5 (1)	98 0 (1)
4	55 0 (2)	107 0 (2)	45 5 (3)	34 0 (4)	39 5 (1)	1 6 5 (1)
5	[illegible]2 0 (3)	4 0 (2)	264 5 (2)	13 5 (1)	6 5 (1)	[illegible]53 0 (2)
Total	333 5 (7)	533 0 (10)	425 0 (11)	244 0 (9)	345 0 (4)	503 5 (7)

Figures in parentheses indicate the number of isolations of Gram negative bacilli

Hexachlorophene preparations should be used *regularly* in order to get maximum effect (8 16 21) This is possibly demonstrated by the tendency of higher counts in hand washings after seven days of use than in the previous counts As the last sampling during treatment was taken on Mondays and the majority of the subjects were off duty on Sundays this probably reflects the less frequent usage of the preparations off duty

The preliminary experiments suggest that treatment with hexachlorophene preparations has a suppressive action on the *transient* flora of the hands This is confirmed by the experiment with inoculation of Staph aureus on the hands after treatment The effect of hexachlorophene preparations used only on duty has been doubted (13 21) However the inoculation experiment demonstrates a considerable effect of residues of antiseptic left on skin after a single thorough hand washing with the preparations Thus it seems desirable that all personnel are instructed to wash their hands thoroughly at the beginning of the day

Gram negative bacilli represent an increasing danger in hospitals Hexachlorophene is less effective against gram negative bacilli and may even sometimes promote colonization of the skin by gram negative organisms (17) In vitro tests of chloroxylenol (1 17) and preparation 1 suggest that these have some effect also against Gram negative bacilli The present investigation however fails to demonstrate any effect on this flora

Detergents with hexachlorophene are often reported to cause some dryness of the skin but allergic reactions seem rare (8 10) The differences in side effects between the preparations demonstrated by this investigation are important when disinfectants for long term use are to be chosen

Table VI *Side effects (skin irritation) of hexachlorophene detergents*

	Preparation no			
Subject no	1	3	4	5
1	0	1	1	0
2	4	0	0	2
3	1	0	0	0
4	2	1	1	1
5	3	1	2	2
6	1	2	1	1
7	2	1	1	1
8	3	0	1	2
9	3	0	1	2
10	2	0	1	3
11	2	0	1	2
12	2	1	1	2
Mean score	2 08	0 58	0 92	1 50

0 = no side-effects
1 = dryness of skin
2 = 1 plus slight irritation (redness *or* soreness)
3 = considerable irritation (redness *and* soreness)
4 = heavy irritation causing interruption of treatment

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REFERENCES

1 Brodie J Hand hygiene Scot med J 10 115 1965
2 Bruun, J N To be published
3 Cox D R Planning of experiments John Wiley & Sons New York 1958
4 Eriksen, K. R. & Lund F Undersøgelser over et kvarternært ammoniumklorids (Rodalon) huddesinfi cerende egenskaber Ugeskr Læg. 115 336 1953
5 Fahlberg, W J., Swan, J C & Seastone C V Studies on the retention of hexachlorophene (G 11) in human skin J Bact. 56 323 1948
6 Joress S M A study of disinfection of the skin A comparison of povidone iodine with other agents used for surgical scrubs Ann. Surg 155 296 1962
7 Juhlin, I & Ericson, C Hospital infections and hospital hygiene at Malmo General Hospital II. Hygienic measures and their correlation with the incidence of infection J Hyg. (Lond) 63 35 1965
8 Lowbury E J L. & Lilly H A., Disinfection of the hands of surgeons and nurses Brit. med J 1 1445 1960
9 Lowbury E J L., Lilly H A. & Bull J P Disinfection of the skin of operation sites Brit. med J 2 1039 1960
10 — Disinfection of hands Removal of resident bacteria. Brit. med J 1 1251 1963
11 — Disinfection of hands Removal of transient organisms Brit med J 2 230 1964
12 — Methods for disinfection of hands and operation sites Brit med J 2 531 1964
13 MacPherson, C R., Sparkman, M F & Whitney D R. Lack of effect of two hexachlorophene-containing soaps under normal hospital working conditions. Amer J Surg. 109 699 1965
14 Mortimer E A., Wolinsky E Gonzaga, A J & Rammelkamp C H Role of airborne transmission in staphylococcal infections Brit med J 1 319 1966
15 Price P B The bacteriology of normal skin a new quantitative test applied to a study of the bacterial flora and the disinfectant action of mechanical cleansing. J infect Dis. 63 301 1938
16 Reber H Bircher J & Grumbach P Zur chirurgischen Handedesinfektion mit Hexachlorophen Schweiz Z. allg. Path 23 581 1960
17 Rubbo S D & Gardner J F A review of sterilization and disinfection Lloyd Luke Ltd London 1965
18 Solberg, C O A study of carriers of Staphylococcus aureus Acta med scand Suppl 436 1965
19 Story P Testing of skin disinfectants Brit med J 2 11 8 1952.
20 Stoughton, R. B Hexachlorophene deposition in human stratum corneum Arch Derm 94 646 1966
21 Weatherall J A C & Winner H I The intermittent use of hexachlorophene soap — a controlled trial J Hyg (Lond) 61 443 1963
22 Williams, R. E O Blowers, R., Garrod L. P & Shooter R. A. Hospital infection. Causes and prevention Lloyd Luke Ltd London 1966

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ATRIAL FIBRILLATION

A Review of 463 Cases from Philadelphia General Hospital from 1955 to 1965

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Abstract The hospital records of 463 patients with atrial fibrillation occurring over a 10-year period at the Philadelphia General Hospital have been reviewed The different etiologic factors have been determined as well as some sexual and racial proportions Criteria of the different groupings are explained and some associated ECG findings reported

Arteriosclerotic and hypertensive heart diseases are responsible for atrial fibrillation in this study in almost 65% of the cases

The incidence of embolic episodes in the different etiologic groups is also compared For the entire series the percentage was 17 9

The purpose of this report is to review some of the pertinent clinical features of atrial fibrillation Particular attention is paid to the probable etiologic background or associated disease concomitant electrocardiographic findings and finally embolic complications Very few authors have reported recently on these aspects of atrial fibrillation although therapy particularly countershock has been extensively discussed

Since atrial fibrillation is a common phenomenon and many of its clinical features change simultaneously with changes in the epidemiology of this arrhythmia a review of these changes is considered justifiable Such a recent epidemiologic study is necessary to evaluate the representativeness of th materials now published in which countershock has been used to convert atrial fibrillation

MATERIAL AND METHODS

The study was based on a review of the hospital records with a diagnosis of atrial fibrillation during a 10-year period (January 1 1955 to December 31 1964) at the Philadelphia General Hospital No cases were included which did not have an ECG tracing available at the time of review which accurately established the diagnosis of atrial fibrillation

The following data were determined in each case age sex race associated and/or etiologic diseases some concomitant ECG findings and finally frequency of embolism

Age

The age of the patient was recorded at time of onset of atrial fibrillation, regardless of whether this occurred before 1955 or during the 10-year period of study

Etiologic factors

Since atrial fibrillation, with few exceptions occurs primarily in elderly patients there was reason to believe that frequently more than one etiologic factor exists Consequently many reports are affected by this drawback However by presenting combination groups of arteriosclerosis and hypertension rheumatic heart disease and arteriosclerosis a more accurate grouping was obtained The criteria of the different groupings in the present study are given below

1 Arteriosclerotic heart disease There was frequently a positive family history of th s disease or evidence of angina pectoris myocardial infarction or diabetes. These patients often presented congestive heart failure

2 Hypertensive cardiovascular disease Hypertension was diagnosed when the blood pres ure readings were at least 100 mm Hg diastolically (1 3) One exception however was a diabetic patient with elevated blood pressure This pat ent was therefore included in group 1 (8 16)

3 Rheumatic etiology was considered in a subject with a history of rheumatic fever or a rheumatic type of infection. Valvular lesions mitral and aortic were present either alone or combined

4 Arteriosclerosis and hypertens on diagnosed as abo e occurring in the same patient

5 Similarly rheumatic heart disease and arteriosclerosis combined in the same pat ent

6 Thyrotoxicosis required a clinical picture of the disease positive tests for thyroid overactivity such as

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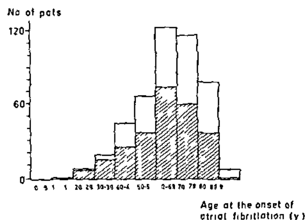


Fig 1 Number of patients in different age groups at time of onset of atrial fibrillation Blank columns denote number of females and hatched columns denote males

Table I *Age distribution of the entire material with atrial fibrillation*

Age groups (y)	Males	Females	Total
10–19		2	2
20–29	7	1	8
30–39	15	3	18
40–49	24	19	43
50–59	36	32	68
60–69	73	48	121
70–79	60	57	117
80–89	36	42	78
90–99	2	6	8
Total no of pat	253	210	463

elevated protein bound iodine increased thyroid uptake of I[131] and an elevated basal metabolic rate

7 Atrial fibrillation associated with syphilitic aortitis This diagnosis was made when there was a classical aortic insufficiency a positive serology test for syphilis and in all cases a history of luetic infection (In two cases the diagnosis was confirmed at autopsy)

8 Alcoholic myocardial disease All these patients had a long history of excessive alcohol intake Often and contrary to beri beri heart disease the food intake in this group of patients seemed to be fairly adequate None of these patients responded to thiamine therapy (5 7 25)

9 Idiopathic atrial fibrillation was considered as the diagnosis in the complete absence of heart disease after extensive medical evaluation (13 20)

10 Severe infections This group was more heterogeneous in composition In our cases the infection was the dominating feature in the patient s clinical picture Gram negative septicemia chronic osteomyelitis lobar pneumonia and extensive tuberculosis involving the heart were the diagnoses in these cases

11 Miscellaneous A few cases were observed with extensive pulmonary neoplasms with pathologico anatomic evidence of spread to the heart There was also one case with atrial fibrillation that started immediately after lobectomy due to pulmonary tuberculosis One young female developed atrial fibrillation during the last trimester of two successive pregnancies without clinical evidence of valvular disease or thyrotoxicosis (19) One case of a congenital heart lesion with a familial rhythm disturbance was discovered (15) One patient with a myocardiopathy probably secondary to lupus erythematosus was also placed in this group A few cases were included with moderate to severe degree of pulmonary emphysema associated with atrial fibrillation

Associated ECG findings

The following findings were recorded myocardial infarctions recent or old complete heart block left and right bundle branch block left and right ventricular hypertrophy The hypertrophy was diagnosed according to the criteria applied by Sokolow and Lyon and in most cases was supported by vectorcardiography (29 30)

Complications with thromboembolic phenomena

Only patients with a well-documented hospital record of an embolic episode were included These emboli occurred either prior to or during the present admission The embolization was divided into cerebral pulmonary and peripheral (leg arm, and abdomen) groups How

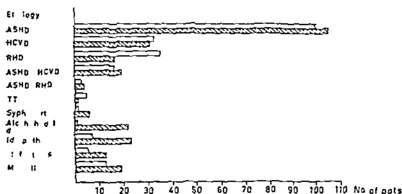


Fig 2 Number of patients in the different etiological groups Blank columns denote number of females and hatched columns denote males

ever the difficulty of antemortem separation of cerebral embolism and thrombosis is well known and should be remembered.

Statistical method

In the statistical analysis the following probability levels of significance were used $p<0.001$ was highly significant * $0.001<p<0.01$ significant * and $0.01<p<0.05$ probably significant The means were given ± the standard error of the mean (1?)

RESULTS

Age sex and race

The entire material consisted of 463 patients The mean age was 65 2±1 0 years There were 253 males and 210 females the ratio of males to females was 1 2 1 0 The mean age of the males was 63 3±1 0 years range 20–91 years and that of females 67 4±1 0 years range 12–97 years (Fig 1 and Table I)

Etiologic factors

The clinical diagnoses obtained are shown in Fig 2 and Table II

Associated ECG findings

Myocardial infarctions were diagnosed in 9 5 % of the entire series Of these 11 4 were acute Left bundle branch block was registered in 7 1 % and complete heart block in 1 1% Left bundle branch block occurred in 10 of the cases with arteriosclerotic heart disease and in 6 3% of the cases with hypertensive cardiovascular disease Left ventricular hypertrophy was observed in 25 1% of the entire series and right ventricular hypertrophy in 2 4 % In the group with rheumatic heart disease however right ventricular hypertrophy was seen in 10 7% and left ventricular hypertrophy in 39 3 % (Table III)

Thromboembolic complications

The incidence of embolization is recorded in Table IV As a percentage of the entire series this was 17 9

DISCUSSION

Comparison with other studies

The size of this series is comparable to some of the larger series that have been previously reported (18 22 28 31) The material consists of only patients admitted and consequently patients

Table II *The different etiologic factors (total numbers races mean ages and percentages)*

Etiology										Mean age									Percentage		
	♂	●♂	♀	●♀	♂+♀	●♂+●♀	♂+●♂	♀+●♀	♂+♀ ●♂+●♀	♂	●♂	♀	●♀	♂+♀	●♂+●♀	♂+●♂	♀+●♀	♂+♀ ●♂+●♀ ±SE	●♂+●♀	♂+♀	♂+♀ ●♂+●♀
ASHD	71	32	63	34	134	66	103	97	200	73 0	69 8	76 2	71 6	74 5	70 8	7 0	74 6	73 3±0 7	33 3	50 7	43 3
HCVD	7	24	19	14	26	38	31	33	64	77 4	61 4	76 0	60 0	75 0	60 9	63 9	69	66 6±1 5	19 1	9 8	13 8
RHD	5	11	15	20	20	31	16	35	51	4 4	47 4	55 7	51 2	57 4	49 8	45 8	53 1	50 8±1 9	15 6	7 6	11 0
ASHD+HCVD	11	8	7	9	18	18	19	16	35	68 5	65 0	73 1	65 2	70 3	65 1	67 0	68 6	67 8±1 7	9 0	6 8	7 8
ASHD+RHD	1	2	2	—	3	2	3		5	29 0	38 5	59 0	—	49 0	38 5	35 3	59 0	44 8±8 7	1 0	1 1	1 1
TT	—	1	1	3	1	4	1	4	5	—	75 0	52 0	68 3	5 0	70 0	75 0	64 3	66 4±6 4	2 0	0 4	1 1
Syph aort	—	5	—	1	—	6	5	1	6	—	68 [illegible]	—	67 0	—	68 0	68 2	67 0	68 0±3 8	3 0	—	1 3
Alcoh heart dis	17	4	1	—	18	4	21	1	22	55 5	50 0	68 0	—	56 2	50 0	54 4	68 0	55 0±2 4	2 0	6 8	4 7
Idiopathic	14	8	—	6	14	14	22	6	28	45 6	37 0	—	43 7	45 6	39 9	42 5	43 7	42 7±2 4	7 0	5 3	6 0
Infectious	9	3	4	—	13	3	12	4	16	62 7	69 3	75 0	—	66 5	69 3	64 3	75 0	67 0± 3	1 5	4 9	3 4
Miscellaneous	10	9	7	5	17	14	19	12	31	61 4	64 6	64 6	45 8	62 7	64 1	64 1	56 8	61 3±2 9	6 5	6 4	6 5
Total	144	107	1 0	92	264	199	51	212	463	64 2	61 2	71 5	61 [illegible]	67 6	61	63 3	67 4	65 2±1 0	100 0	100 0	100 0

Open circles in sex symbols denote Caucasians filled circles denote Negroes
SE – standard error ASHD arteriosclerotic heart disease HCVD – hypertensive cardiovascular disease RHD – rheumatic heart disease TT – thyrotoxicosis Syph aort – syphilitic aortitis Alcoh heart dis – alcoholic heart disease (myocardiopathy)

Table III *Associated ECG findings in three etiologic groups*

Etiology	No of pats	Myocardial infarction (old or new)	LBBB	RBBB	Per cent of recorded ECG findings in entire material
ASHD	200	19 (9 5)	20 (10 0)	1[illegible] (6 0)	25 5
HCVD	64	—	4 (6 3)	5 (7 8)	14 1
RHD	51	—	1 (2 0)	5 (9 8)	11 8

LBBB = left bundle branch block RBBB = right bundle branch block Others as in Table II

dying in the outpatient department and those visiting on an ambulatory basis were excluded

A General Hospital population represents in regard to the socio-economic status of the patients a certain type of selection However this type of hospital population reflects a large proportion of the general population Previous materials were also mainly selected from this type of hospital

Age at onset of atrial fibrillation was similar in my group to that of the material reported on by Sawyer et al (28) The peak incidence in the present study was in the 60–70 age group Earlier reports have shown a lower mean age Most studies report a higher incidence of atrial fibrillation among males than among females The male to f male ratio in my study was 1 2 1

Earlier studies have not reported any racial ratios and therefore a comparison is not possible

Etiology

The difficulties at times of determining a single etiology of atrial fibrillation were evident during this study Particular attention was paid to differentiating between arteriosclerotic and hypertensive heart disease This difficult differential diagnosis is apparent since hypertension has been reported to occur in as high a proportion as 50 % in men and in about 75 % in women with coronary heart disease (16 17) On the other hand hypertensive patients have a high incidence of coronary heart disease Some reports have therefore not separated these diseases but combined them as etiologic factors

In comparison with previous reports there is an expected change in the more recent studies (Table V) (10 18 22 28) The increased role of arteriosclerotic and hypertensive heart disease was observed This trend is probably due to the increased number of patients with arteriosclerotic heart disease as the mean age of the population is increasing Other important factors are the decrease of rheumatic fever and the diminished severity of the disease These changes began in the late 1930s before the antibiotic era (4) As there is a long interval between the attack of

Table IV *Embolic episodes in the different etiologic groups*

Etiology	No of pats	Cerebral emboli	Pulmonary emboli	Peripheral emboli	No of pats with > 1 embolus	No of pats with emboli	Per cent of pats with emboli in entire material
ASHD	200	30	8	4	3	45	22 5
HCVD	64	19	1	—	—	22	34 4
RHD	51	5	4	2	—	11	21 6
ASHD + HCVD	35	5	1	—	—	6	17 1
ASHD + RHD	5	—	—	1	—	1	20 0
TT	5	1	—	—	—	1	20 0
Syph aort	6	—	—	—	—	—	—
Alcoh heart dis	22	—	1	—	—	1	4 5
Idiopathic	28	—	—	—	—	—	—
Infectious	16	—	—	—	—	—	—
Miscellaneous	31	1	—	—	—	1	3
Total	463	61	17	6	3	80	17 9

Abbreviations as in Table II

Table V *The etiology of atrial fibrillation in five series*

Numbers are per cent of total

Authors	Åberg 1968	Sawyer 1956	Goldman 1951	McEachern and Baker 1932	Parkinson and Campbell 1930
No of pats.	463	407	80	575	200
Etiology					
ASHD	43 3	36 1	55 0 (ASHD + HCVD)	31 1	~ 5
HCVD	13 8	9 3		16 9	24 0
RHD	11 0	29 7	31 25	34 4	2 0
ASHD + HCVD	7 8	12 3	—	—	—
ASHD + RHD	1 1	1 5	—	—	—
TT	1 1	7 6	5 0	7 5	14 0
Syph aort.	1 3	—	1 25	3 0	2 5
Miscellaneous	6 5	3 4	7 5	2 1	15 0
Other groups	14 1	0 1	—	5 0	—

Abbreviations as in Table II

rheumatic fever and established rheumatic valvular disease with atrial fibrillation, it is readily accepted that the decrease in rheumatic fever in the late 1930 s and the early 1940 s is responsible for the statistical change at the time of the present study It also explains the higher mean age in the group of rheumatic heart disease in this series compared with earlier studies The clinical view that atrial fibrillation in a young patient is most likely caused by rheumatic heart disease is no longer as valid as in previous generations

The sex ratio is almost identical in both the arteriosclerotic group and the hypertensive group namely 1 1 (Table II) In the arteriosclerotic group there are more males in the comparatively low age groups In the group below 55 years of age there are nine males and three females

As shown in Table II there is a striking difference between the races regarding the most common cause of atrial fibrillation In the white population *hypertensive heart disease* is the cause in 9 8% of the cases but in the Negro it represents 19 1 This difference is highly significant ( ) It is well known that hypertension is seen more frequently and in a more severe form among the Negroes in the United States than among the Caucasians (34) The reverse is true however in the group where arteriosclerosis is the cause In the white group the figure is 50 7% and in the Negro 33 3% This difference is also highly significant ( ) The different epidemiology among the races in the United States has been reported earlier (24) The high incidence of hypertension without arteriosclerosis in this series is surprising Of course there is the possibility of undetected arteriosclerotic heart disease in the hypertensive group The high ratio of Negroes in the series is another explanation

Hyperthyroidism occurred in 1 1% of the cases of atrial fibrillation. This percentage is lower than in earlier reports As most cases with thyrotoxicosis occur during the third and fourth decades of life it is remarkable that the mean age of thyrotoxic heart disease manifested in atrial fibrillation is much higher This observation has led some authors to believe that hyperthyroidism alone does not cause atrial fibrillation without the complication of some additional organic heart disease (27)

A few years ago Brigden and Robertson described the alcoholic myocardiopathy which seems to be a cardiac manifestation of alcoholism other than beri beri heart disease (5 7 25) These authors report rapid atrial fibrillation in half of the patients at some time during the illness The cause might be changes in the distal branches of the coronary arteries particularly those located in the atria (25) The size of this etiologic group will necessarily depend upon the composition of the hospital population My impression is that this group is overrepresented in the series under review No comparison of this group with other studies is possible as the disease has not been recognized as an etiologic factor in earlier reports

Considerable emphasis has been given to the

rarity of atrial fibrillation in syphilitic cardio vascular disease In our series 1 3% of the cases of atrial fibrillation were associated with syphilitic aortitis which is within the same range as in previous reports

Idiopathic atrial fibrillation originally de scribed by Gossage and Hicks in 1913 (11) is more common in males I found a male to female ratio of 4 1 Phillips and Levine (23) and Orgain et al (21) have stated that idiopathic atrial fibril lation can be expected to occur in slightly more than 5% of all cases of atrial fibrillation My result of 6% is in very close agreement with the above prediction

The author observed only one case of atrial fibrillation that occurred during childhood This was a case of transposition of the great vessels in a 12 year-old girl who presented familial congenital atrioventricular dissociation and atrial fibrillation (15) Cookson in his large series of atrial fibrillation consisting of 1164 cases had only 2 5% below the age of 17 and no instances below the age of 12 (6) In 500 cases Thurmann found only four with congenital heart disease as the cause of atrial fibrillation Of these patients three had atrial septum defects either alone or combined with other defects (33)

Thromboembolic phenomena

Embolus formation in atrial fibrillation is often discussed and only in rheumatic mitral disease is there general agreement that fibrillation increases the risk The question of anticoagulation therapy is accordingly controversial in atrial fibrillation due to other causes (2 9)

The high incidence of emboli in the arterio sclerotic and hypertensive groups is noticeable I would suspect that some of the arteriosclerotic hypertensive patients did in fact experience thrombotic rather than embolic obstructions

In the rheumatic group the incidence of embolic phenomen is lower than reported for some series This might be explained to some extent by the fact that the author was unable to make a follow up study The expected higher incidence of cerebral and peripheral emboli in comparison with pulmonary emboli in rheumatic heart disease and atrial fibrillation is not as impressive as in the report by Sawyer et al (28)

In the arteriosclerotic and hypertensive groups there is a surprisingly high occurrence of cerebral and peripheral emboli This is in contrast to reports from necropsy studies in which it has been shown that in arteriosclerotic heart disease there are more thrombi present in the right atrium than in the left (14 32) The possible explanation for the high ratio of cerebral and peripheral emboli to pulmonary emboli is that pulmonary emboli occur more frequently without being diagnosed

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REFERENCES

1 Ask Upmark E A primer of high blood pressure pp 19–24 Scandinavian University Books Stockholm 1968
2 Askey J M Embolism and atrial fibrillation Amer J Cardiol 9 491 1962
3 Björk S Personal communication
4 Bland E F Declining severity of rheumatic fever a comparative study of the past four decades New Engl J Med 262 597 1960
5 Brigden W & Robinson J Alcoholic heart disease Brit Med J 2 1283 1964
6 Cookson H Auricular fibrillation in children. Lancet 2 1139 1929
7 Evans W Alcoholic cardiomyopathy Amer Heart J 61 556 1961
8 Feldman M & Feldman M Jr The association of coronary occlusion and infarction with diabetes mellitus A necropsy study Amer J med Sci 228 53 1954
9 Freeman J & Wexler J Anticoagulants for treatment of atrial fibrillation J Amer med Ass 184 1007 1963
10 Goldman M J Quinidine treatment of auricular fibrillation Amer J med Sci 2 2 382 1951
11 Gossage A M & Hicks J A B On auricular fibrillation Quart J Med 6 435 1913
12 Hill A B Principles of medical statistics 7th ed pp 257–260 Oxford University Press New York 1961
13 Howard E J Chronic atrial fibrillation unrelated to organic diseases follow up study of five cases Amer Heart J 59 343 1960
14 Jordan R A Miller R D Edwards J E & Parker R L Intracardiac mural thrombosis thromboembolism in acute and healed myocardial infarction Circulation 6 1 1952
15 Khorsandian R S Moghadam A N & Muller O F Familial congenital A V dissociation Amer J Cardiol 14 118 1964
16 Levy H & Boas E P Coronary artery disease in woman J Amer med Ass 107 97 1936

17 Mann, G V The epidemiology of coronary heart disease Amer J Med 23 463 1957
18 McEachern D & Baker B M Auricular fibrillation its etiology age incidence and production by digitalis therapy Amer J med Sci 183 35 1932
19 McMillan T M & Bellet S Ventricular paroxysmal tachycardia Report of a case in a pregnant girl of 16 year with apparently normal heart Amer Heart J 7 70 1931
0 Neufeld H Wa envoort C A Burchell H B & Edwards J E Idiopathic atrial fibrillation Amer J Cardiol 8 193 1961
71 Orgain, E S Wolfe L & White P D Uncomplicated auricular fibrillation and auri ular flutter Arch intern Med 57 493 1936
Parkinson J & Campbell M Paroxysmal auricular fibrillation Quart J Med 24 67 1930
73 Phillips E & Levine S A Auricular f brillation without other evidence of heart disease Amer J Med 7 478 1949
24 Phillips J H Jr & Burch G E Card ovascular diseases in the white and Negro races Amer J med Sci 738 97 1959
25 Pintar K Wolanskyj B M & Gubbay E R Alcoholic cardiomyopathy Canad Med ass J 93 103 1965
26 Root H F Bland E F, Gordon, W H & White P D Coronary atherosclerosis in diabetes mellitus J Amer med Ass 113 27 1939
27 Sandler G & Wilson G M The nature and prognosis of heart disease in thyrotoxicosis A review of 150 patients treated with I[131] Quart J Med 78 347 1959
28 Sawyer C G Bol n L B Stevens E L Daniel L B Jr O Neill N C & Hayes D M Atrial fibrillation Its etiology treatment and association with embolization Sth med J (Bgham Ala) 51 84 1958
79 Sokolow M & Lyon T P The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads Amer Heart J 37 161 1949
30 — The ventricular complex in right ventricular hypertrophy as obtained by unipolar precordial and limb leads Amer Heart J 38 273 1949
31 Stroud W D Laplace L B & Reisinger J A The etiology prognosis and treatment of auricular fibrillation Amer J med Sci 183 48 1932
32 Soderstrom N Myocardial infarction and mural thrombosi in the atria of the heart Acta med scand Suppl 217 1948
33 Thurmann, M Coarse atrial fibrillation in congenital heart disease Circulation 32 290 1965
34 Weiss M M & Prusmack J J Essential hypertension in the Negro Am r J med Sci 195 510 1938

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DIRECT CURRENT CONVERSION OF ATRIAL FIBRILLATION—LONG TERM RESULTS

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Abstract During a 4-year period (August 1963 to September 1967) 181 patients with atrial fibrillation were treated with direct current countershock technique

The patients have been followed during 5 to 5 months The immediate and the long term results are reported The long term results are disappointing only [illegible] % of those reverted remain in sinus rhythm after one year Factors which might influence the result are studied Finally indications and contra indications for conversion of atrial fibrillation are discussed

Direct current countershock has become a valuable method in the treatment of some arrhythmias Since Lown et al introduced the method (19) it has gained widespread use Conversion of atrial fibrillation to sinus rhythm is desirable for many reasons The hemodynamic effectiveness of the heart is generally improved especially during exercise (10 26 33 35 37) Further it is generally accepted that restored sinus rhythm decreases the considerable risk of systemic and pulmonary embolism, particularly in rheumatic heart disease (39)

Synchronized DC shock is a safe and effective method to restore atrial fibrillation to sinus rhythm (1 4 7–9 11 13 16–20 23–25 27–29 34) Thus the decisive factors whether or not an attempt should be made to convert atrial fibrillation will mainly be the estimated risks and the possibility of maintaining sinus rhythm As with all new therapeutic methods thorough follow up studies are necessary to define the indications

The aim of this paper is to report on our long term results after DC conversion of atrial fibrillation

MATERIAL AND METHODS

From August 1963 to August 1967 181 patients with atrial fibrillation have been treated with DC-shock in our department The pertinent clinical data on the material are shown in Table I

The patients with arteriosclerotic heart disease had a history of angina pectoris earlier myocardial infarction hypertension or diabetes Only one of these patients was under 50 years of age

The diagnosis of rheumatic valvular disease was established by cardiac catheterization angiocardiography and/or surgery in 81 5 and based on clinical findings in 18.5 %. Patients with severe mitral stenosis and other valvular diseases were not converted prior to surgery Of the 68 patients with mitral commissurotomy 57 % were converted 4–8 weeks after surgery A ball valve prosthesis was inserted in 11 cases

In the miscellaneous group two patients had ventricular septal defects, one patent ductus arteriosus one coronary artery aneurysm, and one hypertrophic subvalvular aortic stenosis In one patient the ventricular septal defect was combined with pulmonary stenosis All these patients underwent corrective surgery prior to conversion.

The diagnosis of myocarditis was based on the arrhythmia, the sudden development of cardiac enlargement and heart failure in the absence of valvular and arteriosclerotic heart disease

Four patients had persistent atrial fibrillation after successful treatment of hyperthyroidism Patients with hyperthyroidism were not treated for atrial fibrillation until they had become euthyroid

The remaining group was diagnosed as "idiopathic" when there was no evidence of heart disease

The duration of atrial fibrillation, prior to the DC shock, has been determined as the time during which the patient definitely noticed an irregular heart activity If the patient was unable to give a date we have recorded the first time the arrhythmia was diagnosed by a physician

Many patients have been converted several times The result of the patient's first conversion or where two or more attempts were made during the same admission, the last one has been tabularized

The technique used for this material, has been previously reported (1 7) All conversions were performed on hospitalized patients. The patient was in a supine position. A short acting barbiturate (Pentothal®) intravenously administered was used as an anesthetic Digitalis was discontinued 48–72 hours before the DC-shock

Table I *Total group of patients with atrial fibrillation where conversion was attempted*

Etiology	Number of cases ♂	♀	Total	Age (y) Mean	Range	Heart volume (ml/m² BSA) Mean	Range
ASHD	28	17	45	62	48–73	601	410–790
MS							
Operated	11	28	39	49	32–63	633[a]	470–810
Not operated	3	3	6	57	43–68	616	415–750
MI							
Operated	1	3	4	44	37–53	665	550–850
Not operated	3	1	4	50	35–64	735	600–940
MS + MI							
Operated	8	8	16	46	36–55	672	445–950
Not operated	6	9	15	52	44–62	675	480–1110
AS – AI							
Operated	2	1	3	50	42–58	747	640–900
Not operated	1	—	1	—	60	—	680
M + A							
Operated	8	14	22	46	37–56	714	480–1000
Not operated	3	3	6	51	29–63	765	560–1020
Miscellaneous	2	6	8	48	34–60	736	590–870
Myocarditis	5	—	5	48	41–54	654	5 0–760
Treated hyperthyroidism	—	4	4	64	58–71	560	470–630
Idiopathic	3	—	3	41	40–44	483	440–510
Total	84	97	181	5.	29–73	693	410–1110

[a] One patient s heart volume not known

Abbreviations ASHD = arteriosclerotic heart disease MS = mitral stenosis MI = mitral insufficiency AS – AI = aortic stenosis and/or aorti insuffi iency M + A = mitral and aortic lesions

Until late 1966 quinidine was given a few days prior to conversion In most cases we used the Cardiac Synchronizer (Corbin Farnsworth Company) One shock *was given at each of the energy levels 80 100 200 300* and up to 3 attempts at 400 Ws This procedure was not strictly followed in all cases

With regard to maintenance therapy quinidine was given as a rule until the beginning of 1967 when other investigations were started in an attempt to develop a better prophylactic therapy These will be published else where (?) The quinidine dose was adjusted according to the serum level of quinidine (12) aiming at a concen tration of 5–7 mg/l

The roentgenological heart volume was measured by the method de crib d by Jonsell (14) during the same admission when the conversion was performed

RESULTS

Immediate results

In 181 patients 290 attempts at conversion were made Sinus rhythm for a period of at least 30 seconds was obtained in 146 patients The con vertibility rate was 81 2%

There were more failures among patients with atrial fibrillation of long duration On the other hand there is no difference between this group of failures and those converted in regard to heart volume or age (Tables III and IV) In the valvular disease group there are only four failures out of 20 with a heart volume exceeding 750 ml/m BSA These four patients had atrial fibril lation of long duration 13 10 3 and 1 1/2 years respectively

The total number of shocks administered was 486 In the group with successful conversions the number of shocks on each occasion was not sig nificantly related to sex age etiology or main tenance of sinus rhythm after the conversion When comparing different body weights a trend is apparent patients weighing more required more shocks The difference however is not sig nificant

Long term results

The follow up period varied from 5 months to 52 months Three patients out of 181 did not take part in the follow up Two of these could not be reached One of them was a Polish citizen The third patient had sinus rhythm 6 1/2 months after conversion but was later killed in a traffic accident

Table II *Persistence of sinus rhythm after conversion in relation to etiology*

Etiology	Total	Failures	Period with sinus rhythm <24 h	24 h–1 mo	1–3 mo	3–12 mo	>1 y
			No of patients				
ASHD	44	11	7	7	6	6	7
MS							
Operated	39	4	5	14	3	10	3
Not operated	6	—	—	—	—	3	3
MI							
Operated	4	2	—	—	1	—	1
Not operated	4	—	1	—	—	1	2
MS+MI							
Operated	16	3	2	6	1	—	4
Not operated	15	6	1	6	—	1	1
A−M+A							
Operated	24	5	2	9	2	3	3
Not operated	7	1	—	—	2	3	1
Miscellaneous	7[a]	—	—	2	1	—	4
Myocarditis	5	1	—	1	—	2	1
Treated hyperthyroidism	4	2	1	—	—	—	1
Idiopathic	3	—	—	2	—	—	1
Total	178	35	19	47	16	29	32

One patient lost to follow up
Abbreviations A − M + A = aortic or aortic and mitral valvular d sease Others as in table I

Twenty-eight of 178 patients still remain in sinus rhythm with an average follow up period of 1 year 6 months and 10 days range 5 months to 36 months Table II shows the persistence of sinus rhythm after conversion in the different etiologic groups in Table III it is related to the duration of atrial fibrillation and in Table IV to the heart volume It is apparent that the dura tion of the fibrillation is of utmost importance in practically all etiologic groups On the other hand the heart volume seems to be less impor tant as a cause of relapse The high frequency of relapse in operated mitral stenosis is evident.

More than one attempt at conversion was

Table III *Persistence of sinus rhythm after conversion correlated with diagnosis and duration of atrial fibrillation*

Etiology	Total	Failures S	Failures M	Failures L	<1 mo S	<1 mo M	<1 mo L	1–3 mo S	1–3 mo M	1–3 mo L	3–12 mo S	3–12 mo M	3–12 mo L	>1 y S	>1 y M	>1 y L
ASHD	41 *3	1	4	6	3	5	4	4	1	1	1	1	3	3	-	2
MS	41 *4	—	—	4	3	2	13	1	1	—	1	4	7	—	-	3
MI	8	—	—	2	—	—	1	—	—	1	—	—	1	3	—	—
MS + MI	30 *1	1	1	7	5	2	8	—	—	1	—	1	—	1	—	3
A − M + A	31	—	1	5	2	4	5		—	2	6	—	—	1		1
Miscellaneous	6	—	—	—	1	—	—	—	1	—	—	—	—	—	?	2
Myocardit s	5	1	—	—	1	—	—	—	—	—	1	—	1	—	1	—
Treated hyperthyroid sm	4	—	1	1	1	—	—	—	—	—	—	-	—	—	1	—
Idiopathic	3	—	—	—	1	1	—	—	—	—	—	—	—	—	1	—
Total	169	3	7	5	17	14	31	7	3	5	9	6	12	8	11	11

An asterisk preced ng a number denotes the number of patients with unknown duration of atrial fibr llat on
Abbre iations S = short = < 6 months. M = med um = 6–24 months. L = long = > years. Others as in Tables I and II

Table IV *Persistence of sinus rhythm after conversion correlated with diagnosis and heart volume*

		Period with sinus rhythm														
		Failures			< 1 mo			1–3 mo			3–12 mo			> 1 y		
Etiology	Total	S	M	L	S	M	L	S	M	L	S	M	L	S	M	L
ASHD	44	1	8	2	3	10	2	1	4	—	2	4	—	3	4	—
MS	44 [1]	—	4	—	2	12	4	1	2	—	5	7	1	4	1	1
MI	8	—	2	—	—	1	—	—	1	—	—	—	1	—	1	[illegible]
MS + MI	31	1	5	3	2	10	3	—	—	1	1	—	—	[illegible]	2	1
A – M + A	30 [2]	—	3	2	1	6	3	—	[illegible]	3	—	3	2	—	5	—
Miscellaneous	7	—	—	—	—	1	1	—	1	—	—	—	—	—	2	2
Myocarditis	5	—	1	—	—	1	—	—	—	—	—	1	1	1	—	—
Treated hyperthyroidism	4	1	1	—	—	1	—	—	—	—	—	—	—	1	—	—
Idiopathic	3	—	—	—	2	—	—	—	—	—	—	—	—	1	—	—
Total	176 [3]	3	24	7	10	42	13	2	10	4	8	15	5	1[illegible]	15	6

An asterisk preceding a number denotes the number of patients who did not have a chest X ray on the same admission as the conversion
Abbreviations S = small = < 550 ml/m² BSA M = medium = 550–750 ml/m² BSA L = large = > 750 ml/m² BSA Others as in Tables I and II

made in 68 cases of which 66 could be followed In seven cases sinus rhythm was never obtained In 36 of the remaining 59 cases no attempt resulted in sinus rhythm lasting longer than 3 months Only 12 cases were in sinus rhythm after the last attempt with an observation period of 2 months to 2 ¹/ years and where 33 conversions had been performed More than three conversions had been performed in nine cases It is of interest that only in six cases was there a major difference in the outcome of the conversions as regards persistence of sinus rhythm

One hundred and six patients received quinidine as maintenance therapy In three cases the maintenance dose was not adequate and six patients discontinued the drug either of their own accord or on a physician s recommendation Of the 97 patients who received adequate quinidine treatment only 27 % remained in sinus rhythm after 3 months

Table V *Long term results in different reports*

Authors	No of pats converted to sinus rhythm	*Convertibility rate* ( )	*Proportion valvular disease* to others	*Percentage remaining in sinus rhythm at different times* 1 mo	2 mo	3 mo	4 mo	1 y	2 y
Rabbino et al (31)	31	88	3 3 1		32				
Korsgren et al (18)	107	78	1 4 1				40		
Killip and Yormak (17)	115	87		60				27	
Halmos (8)	137	78	4 0 1			55		(4)	
Hurst and Logue (13)	121	96[b]	0 5 1			87			
Morris et al (27)	100	93	1 9 1		65			58	
Eberdt et al (8)	35	88	1 6 1	57		46			
Futral and McGuire (9)	42	85	2 1 1			45			
Wikland et al (40)	60[d]	81	0 6 1	60				25	
Radford and Evans (32)	119	77	1 2 1			47		18	13
Present study	146	81	2 1	55		4		22	11

Follow up period 9 months instead of 1 year
[b] Convertibility rate measured for many attempts
[c] This number stands for 117 patients who tolerated maintenance quinidine therapy Furthermore some patients reverted without shock
[d] Eight patients had atrial flutter

The patients who did not receive quinidine were discharged without therapy in two cases and were given procainamide in 13 cases In this small combined group 47% remained in sinus rhythm after 3 months We have also studied the whole material with regard to earlier conversions with quinidine Sixty three patients out of 181 had previously been reverted by quinidine There were 12 cases where DC shock failed but quinidine had earlier proved successful However it is not possible to draw any conclusions since there was a time lapse between the different attempts with the possibility of deterioration of the heart disease

Complications

These will not be discussed as they have recently been published by our department (1) Only one point should be stressed During the first 3 years we had eight cases with ventricular tachycardia or ventricular fibrillation in immediate relation to the shock Since one and a half year ago when we abandoned administration of quinidine prior to the DC shock we have not had a single case with this severe complication

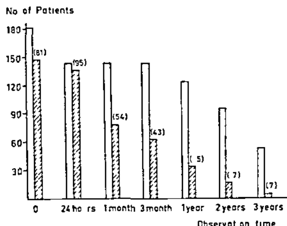


Fig 1 Number of patients in sinus rhythm after different follow up times correlated to total number at risk. Blank column denotes total number of patients at risk at different observation periods Hatched column denotes total number still in sinus rhythm Number within brackets is the percentage of patients remaining in sinus rhythm, correlated to total number of patients after that observation period

DISCUSSION

Our case material is not representative of an unselected sample of atrial fibrillation (3) There is a predominance of patients with operated heart disease of both rheumatic and congenital etiology This is du to the active thoracic surgery performed in Uppsala during the time of this study In Table V the composition of the diagnoses is compared with that of other DC series

The mean duration of atrial fibrillation in our series 2 years and 10 months is in good agreement with that of other authors (18 22 28 34 40) It is important to recognize this as on the whole there seems to be a correlation between the duration of fibrillation and the long term result

The percentage of successful conversions 81 2 is approximately the same or slightly lower than that in other series (8 9 22 27 28) The differences mainly reflect the various criteria used for successful conversion In some series the convertibility rate is lower but there as a rule conversion was only regarded as successful when sinus rhythm was maintained the following day (18 34) On the other hand there was a very low frequency of relapses during the first 24 hours (Fig 1) Another explanation might be the time when maintenance therapy was started Rossi and Lown (36) have recently reported on the benefits of starting quinidine prior to the shock Others believe as we do that there might be more arrhythmias at conversion if quinidine is already administered (1 6) Thus it is possible that the first method increases the convertibility rate but causes more complicating arrhythmias

With regard to *immediate results* some authors state that patients requiring high energy levels are less stable in sinus rhythm Oram and Davies report on ten patients where 400 Ws were used and none was converted to sinus rhythm (28) Korsgren et al found that no patient requiring 300 Ws or more maintained sinus rhythm at the end of th ir follow up study (18) In our material 32 patients maintained sinus rhythm for more than one year and nine of these required 400 Ws We believe that, if a first or second attempt at conversion is indicated then it is necessary to *try also high energy levels (40) We do not consider* that it is possible to predict the prognosis from the energy levels used for conversion

In our material we have not found any sig

nificant difference in the energy required between groups which differ in etiology age or sex This is in accordance with the findings of many authors (18 27 34) but contrary to Oram and Davies who reported lower convertibility in patients over 50 years of age (28)

Weight seems to influence the energy level required patients weighing more require more energy But the difference in the weight classes investigated is not significant We are not aware that this has been previously discussed The explanation might be a decreased myocardial current

In the group with unsuccessful conversions there was a fairly high percentage of patients with arteriosclerotic heart disease The group of failures however is not different from the whole group of patients with arteriosclerotic heart disease in age and heart volume but only in the duration of atrial fibrillation There are more patients with a long duration in the failure group

With regard to the *long term results* the group with arteriosclerotic heart disease does not differ in any essential respect from the groups with valvular disease This confirms some earlier reports (8 18 27)

The group with mitral stenosis as the sole lesion is doing better than the groups with combined valvular lesions as might have been expected and as has also been previously demonstrated (5 22 25 28) We have already mentioned the high frequency of relapse in operated mitral stenosis which is in accordance with some other studies (18 31)

A tight mitral stenosis should not be converted prior to surgery (15 18 22) After mitral commissurotomy or other corrective surgery the countershock should not be administered until after several weeks (15 27) With regard to the last statement no results are given in this report but this is supported by a considerable number of patients in our department of thoracic surgery who were given countershock in the immediate postoperative period In nearly every case the patient relapsed into atrial fibrillation shortly afterwards

In the group with treated hyperthyroidism combined with atrial fibrillation we had two failures out of four patients In spite of the group being small the poor result is noticeable Divergent results have been reported (11 30 32 34)

As a whole the long term results after DC conversion have been disappointing and are identical with those from the quinidine era (38) At least somewhat better results could have been expected owing to the generally higher reversion rate and to the fact that quinidine sensitive patients could be treated Some long term results from other studies are shown in Table V Comparisons of this kind are naturally complicated by variations in selection of material and duration of follow up The problem that still needs to be solved is how to achieve effective maintenance therapy to avoid recurrence of the arrhythmia. In this respect conditions are not better today than they were years ago At present we do not know whether quinidine or other antiarrhythmic drugs are of any value in preventing relapse in cases of atrial fibrillation (2 18 28)

According to our experiences as well as those reported by other investigators some pertinent indications for conversion of atrial fibrillation might be proposed

I In patients with atrial fibrillation of recent date or extending up to a duration of some years DC conversion should be attempted All energy levels should be used if needed Practically no age limit exists this applies particularly to intractable cardiac decompensation

After a relapse a new attempt is recommended if the first was successful and the patient maintained sinus rhythm sufficiently long to show that clinically his condition had undoubtedly improved If the first attempt was unsuccessful according to the above definition a second attempt might be made but a better result this time is to be considered rather as an exception.

II In all patients with atrial fibrillation and episodes of embolization

III In patients with atrial fibrillation combined with a disease that can be cured or improved e g by surgery no conversion should be performed prior to such cure or improvement. Consequently a patient with acute myocarditis and atrial fibrillation should not be converted until the infection is healed A hyperthyroid patient should not be converted before becoming euthyroid In patients with multivalvular disease without tight mitral stenosis on rare occasions, conversion prior to surgery might be indicated in an attempt to improve the patient s condition

for the surgical procedure Another exception might be atrial fibrillation occurring in the course of an acu e myocardial infarction where some authorities believe that immediate conversion is indicated

IV After corrective surgery of any heart le sion atrial fibrillation should not be converted until at least 6–8 weeks postoperatively

There are few *absolute contraindications* for its use today and these seem to be rather universal (*a*) Digitalis intoxication or arrhythmias due to digitalis It is possible that quinidine intoxication also should be included here (1 31) (*b*) Atrial fibrillation with complete heart block where no hemodynamic improvement is possible by means of conversion (*c*) Patients with a recent thrombo embolic episode should not be converted before anticoagulant therapy has been applied (*d*) Tight mitral stenosis

Among more *relative contraindications* can be listed longstanding atrial fibrillation severe mitral regurgitation electrolyte imbalance (espe cially hypopotassemia) and finally atrial fibril lation in elderly patients without symptoms

CONCLUSION

During a 4 year period (August 1963 to Septem ber 1967) 181 patients with atrial fibrillation were treated with DC shock The convertibility rate was 81 The long term results are pre sented In the total material only 42 main tained sinus rhythm 3 months after conversion and 22° after one year in spite of prophylactic quinidine medication in most cases The long term results are related to the duration of atrial fibrillation before conversion whereas age and heart volume do not influence the prognosis to any great extent

The number of shocks given in various patient groups was studied There was no significant dif ference with regard to age sex and etiology There seems to be a definite trend in regard to weight patients weighing more required more energy though the difference was not statistically significant

If conversion is indicated we believe that one should not refrain from using high energy levels We found that among the patients who remained in sinus rhythm for one year after conversion as many as 28 1° needed one or more shocks with an energy of 400 Watt seconds This is contrary to the findings of some authors who claim that to some extent prognosis might be predicted from the energy level used for conversion

In many of our cases repeated conversions were performed on the same patient It was an exception only when a later conversion gave a better long term result than the first attempt

Finally the indications and contraindications for conversion are discussed on the basis of both our experience and that of other investigators

REFERENCES

1 Aberg H & Cullhed I Direct current countershock complications Acta med scand 183 415 1968
2 Aberg H & Cullhed I Prokainamid och kinidin som profylaktikum mot recidiv av formaksflimmer Nord Med 79 781 1968
3 Aberg H To be published
4 Aslaksen, B B & Gjemdal T Elektrisk regulering av atrieflimmer T norske Lægeforen 87 25 1967
5 Bell, H Pugh D & Dunn, M Failure of cardio-version in m tral valve disease Arch. intern Med 119 257 1967
6 Castellanos A Jr Lemberg L & Johnson D Countershock exposed quinidine syncope Amer J med Sci 250 254 1965
7 Cullhed I Holmdahl M H son & Malers E Ex tern likstromschock vid supraventrikulara arytmier Svenska Lak Tidn 61 742 1964
8 Eberdt E C Brill I C & Rogers W R Value of cardioversion in chronic atrial fibrillation Arch. in tern Med 119 253 1967
9 Futral A A & McGuire L B Reversion of chron c atrial fibrillation J Amer med Ass 199 885 1967
10 Graettinger J S Carleton, R A & Muenster J J Circulatory consequences of changes in cardiac rhythm produced in patients by transthoracic direct current shock. J clin. Invest 43 .290 1964
11 Halmos, P B Direct current conversion of atrial f'brillation Brit Heart J 8 30' 1966
1' Hamfelt A & Malers E Determination of quini dine concentration n serum in the control of quini dine therapy Acta Soc Med Upsalien. 68 181 1963
13 Hurst J W & Logue R B The heart, p 316 The Blakiston Division McGraw Hill New York 1966
14 Jonsell S Method for the determination of the heart size by teleroentgenography (A heart volume index) Acta radiol (Stockh) .0 3.5 1939
15 Kerth W J Selzer A Keyani K & Gerbode F The electr cal con ersion of cardiac arrhythmias J cardio asc Surg 5 1' 1964
16 Killip T Synchronized DC precordial shock for arrhythm as Safe new techn que to establish normal rhythm may be utilized on an elective or on emergency bas s J Amer med Ass 186 1 1963
17 Killip T & Yormak, S Short and long term re sults f om direct current conversion for atr al f bril

Table I *Cholesterol esters*

Value in mg per 100 ml of plasma if not otherwise indicated

Subject	Sex/Age	Total lipid	Type[c]	Cholesterol Total	Cholesterol Ester[d]	Cholesterol/phospholipid ratio	Fatty acid composition, value in per cent of total: 12 0[e]	14 0	14 1	16 0	16 1	17 0	17 1	18 0	18 1	18 2	18 3	00
							—	0 5	Tr[k]	12 5	4 3	—	0 2	0 9	2? 9	47 9	0 4	0 5
							—	0 4	—	2 5	1 1	—	0 2	0 2	6 1	6 1	0 4	0
O N	♀/3?	820	II	318	308	1 0	—	—	—	10 2	2 4	—	—	0 8	20 4	34 4	Tr	—
		856		320	340	1 1	—	Tr	Tr	8 8	2 4	—	Tr	0 9	21 6	36 9	Tr	Tr
		690		2?7	246	0 9	—	0 9	Tr	16 1	5 9	—	Tr	0 9	26 8	37 8	0 4	Tr
K L	♀/48	972	II	350	380	1 0	—	0 7	Tr	15 3	6 8	—	Tr	0 8	29 0	42 5	Tr	Tr
		816		279	277	0 9	—	0 5	—	8 0	3 3	—	—	0 3	14 6	26 6	Tr	Tr
		798		?97	274	0 9	—	Tr	—	8 7	3 3	—	—	0 6	2? 1	40 7	Tr	Tr
		754		246	253	0 8	—	Tr	—	12 3	4 9	—	—	Tr	23 9	55 1	—	Tr
		676		230	237	0 8	—	Tr	—	10 8	5 9	—	—	0 4	27 8	46 8	Tr	Tr
H T	♀/63	914	II	372	383	1 1	—	0 6	Tr	14 3	7 3	—	Tr	0 3	?6 7	46 7	Tr	Tr
		554		236	230	1 2	—	0 8	Tr	13 4	5 7	—	Tr	0 5	9 7	46 1	Tr	Tr
		558		190	239	0 9	1 8	1 4	Tr	11 6	8 3	—	—	Tr	25 1	46 4	Tr	—
		570		178	247	0 9	Tr	—	—	10 3	5 4	—	—	Tr	24 8	47 5	Tr	Tr
K F	♂/55	1284	II	475	497	1 1	—	0 8	Tr	11 4	5 8	—	Tr	0 9	20 6	34 ?	0 5	Tr
		708		258	311	1 2	—	—	—	10 4	5 0	—	—	1 0	31 2	46 9	Tr	Tr
S B	♀/59	1758	II	790	1030	1 8	10 8	1 6	—	9 7	3 6	—	—	0 9	22 1	40 5	—	Tr
		696		194	309	0 8	—	—	—	11 7	5 3	—	—	Tr	2? 2	47 1	—	Tr
E B	♀/68	940	II	392	403	1 5	—	Tr	Tr	14 6	6 7	—	—	1 3	28 6	44 3	Tr	Tr
		908		35?	430	1 3	—	Tr	—	9 8	3 7	—	—	1 0	22 2	51 4	Tr	Tr
A	?/32	1080	V	438	447	1 4	2 1	Tr	Tr	11 8	3 1	—	—	1 3	?6 0	35 9	—	Tr
		8?8		335	373	1 4	—	0 8	Tr	15 5	7 5	—	Tr	1 9	27 4	36 0	—	Tr
		740		248	292	1 2	—	Tr	Tr	10 4	4 4	—	0 3	0 9	???	30 2	—	1 8

[a] Normal value 13 women and 18 men [b] Standard deviation According to Fredrickson s system of phenot ping hyperlipoproteinemias [d] Total amount of cholesterol esters [e] The cipher preceding indicates the number of C atoms in the fatty acid The cipher following indicates the number of double bonds in the molecule [f] Unidentified components are grouped according to their retention values (T_R value) related to the retention value for 18 0 (=1 0)

the patients had primary myxedema two had typo thyroidism after strumectomy for toxic goiter One patient had become hypothyroid while being treated with thiamazolum (NFN) and another had acute strumitis

The normal values for the different fatty acids were obtained from 19 men and 1? women aged ?5–71 years (mean age 48 years) all euthyroid and with no known disease involving lipid metabolism

Blood samples were taken after the patient had fasted for 1?–14 hours Potassium-ethylene diamine tetra acetic acid (K EDTA) was used as a stabilising agent The blood samples were taken from both hospitalized patients and outpatients There is no reason to suppose that the hospital diet was different from the patients average food After collecting the samples the plasma was immediately separated by centrifugation If the examination was not carried out within a few hours the samples were stored in a refri erator at +4 C

Lipoprotein electrophoresis was performed on paper at room temperature in barbital buffer with ionic strength of 0 05 pH 8 6 containing 1 albumin (human) and 0 001 *M* EDTA (14) for 2 /? hours at ? 0 \ (about 4 ma per strip) For staining Oil Red O was used according to Jencks and Durrum (1?) The lipoprotein

T_R[f] 2 44– ? 56	T_R 2.83– ∠93	20 3 (? 0)	20 4 (2 1)	T_R 4 30– 4 85	20 5	T_R 6 5– 7 0	T_R 9 5–11 0 / 22 6	Remarks
—	—	0 6	4 5	—	—	—	4 7[a]	
—	—	1 3	1 7	—	—	—	4 7[b]	
0 8	5	—	2 8	—	—	—	? 8	Hypothyr Strumectomy sequelae PBI[g] 1 5 BMR 8
—	0 9	0 4	4 5	—	—	13 6	10 0	Treatment started PBI 1 7
—	—	0 7	10 4	—	—	—	Tr	Now euthyr treated with desiccated thyr PBI 5 4 Se thyr[h] 6 0
—	—	—	4 9	—	—	—	—	Primary myxedema. PBI 0 3 BMR 60–77
2 0	5 0	—	1 4	—	—	—	38 2	For 3 weeks treated with desiccated thyr 100 U a day PBI 0 9 BMR 91
—	—	—	4 6	Tr	—	—	20 0	For 2 months treated with desiccated thyr
—	—	—	3 8	—	—	—	Tr	For 4 months still hypothyro d BMR 84 PBI 1 4
—	—	—	3 1	—	—	—	5 1	Now nearly compensated PBI 3 ? BMR 89
—	—	—	3 9	—	—	—	—	Primary myxedema. PBI 3 7 Se thyr 1 7 T_3[i] 5 6
—	—	—	3 8	—	—	—	Tr	Treated for 2 weeks with desiccated thyr PBI 3 2 T_3 6 1 Se thyr 0 9 BMR 81–85
—	—	—	4 4	1 0	—	—	—	Treated for 4 weeks with desiccated thyr BMR normal PBI 3 2 T_3 7 3 Se thyr 2 0
—	—	—	5 1	—	Tr	—	6 9	Treated for 10 weeks with desiccated thyr PBI 4 0 T_3 8 7 Se thyr 4 9 Euthyr
—	—	0 9	4 6	Tr	—	Tr	0 3	Primary myxedema Se thyr 1 7
—	—	—	5 4	—	—	—	—	For 1 month treated with desiccated thyr Clinically compensated T 8 6 Se thyr ? 9
—	—	—	5 0	Tr	—	—	5 6	Strumectomized 0 years ago BMR 58 Se thyr 0 9 Iodine treated
—	—	—	6 2	Tr	—	—	7 5	Now treated with des ccated thyr for ∠ months Se thyr 6 7 PBI 4 3
—	—	—	3 4	—	1 1	—	—	Hypothyr after Thycapzol treatment. PBI 2 2 BMR 86 Se thyr 3 1
—	—	—	5 0	1 6	—	—	5 2	Thycapzol discontinued for 1 month PBI 3 8 Se thyr 6 3
—	—	—	6 6	—	—	—	13 1	Diab mell for 15 years Now acute strum tis. PBI 1 4 Se thyr 2 3
	—	—	6 8	—	—	—	4 1	1 month later still hypothyr – treatment PBI 1 0 Se thyr 0 T_3 10 0
—	—	3 1	6 1	0 3	—	—	0 0	2 months later still hypothyr T_3 8 8 PBI 0 6 Se thyr 1 1

[g] PBI = protein bound iodine *in μg per 100 ml Normal values 3 5–7 5 μg per 100 ml* [h] *Se thyr = serum thyroxine in* μg per 100 ml Normal values 4 5–13 5 μg per 100 ml [i] T = I labelled tri odothyron ne uptake in the erythrocytes Normal value 6 5–1 3 [k] Tr = trace

pattern was classified as su gested by Fredrickson et al (9)

Plasma total cholesterol was determined according to Runde (?0) *Plasma triglycerides* were determined chemically (6) as well as by column chromatography described below *Total plasma lip d* was estimated gravimetrically after Folch et al (8)

The lipid f actions were separated by silicic acid column chromatography (19) into three fract ons 1 Cholesterol esters triglycerides, free cholesterol and free fatty a ids and 3 phosphol pids The small amount of non-esterified fatty acids in the second fraction was not taken into cons deration.

Gas liquid ch omatog aphy To estimate the fatty acid pattern of the three main l pid fractions the methylated fatty acids were subjected to gas liqu d chromatography as described by Jesting and Bang (13) using a Pye Argon Chromatograph at 188 C with an ethylen glycol succinate column Argon flow was 30 ml/min Detector Strontium 90 beta ionisation

Thin layer ch omatography In order to separate the different fatty acids according to their number of double bond in certain cases the fatty acid methyl esters were subjected to thin layer chromatography on a $AgNO_3$-enr ched silica gel layer (17) prepared by us ng a L 5 (w v) solution of AgNO nstead of water in the preparat on of the plates The methylated fatty acids were appl ed to the pla es in a band dissolved in petrol

Table II *Gl)cerides*

Value in mg per 100 ml of plasma if not otherwise indicated

			Fatty acid composition value in per cent of total																	
Subject	Sex/Age	Glycerides	1 0	14 0	14 1	16 0	16 1	17 0	17 1	18 0	18 1	18 2	18 3	20 0	T_R 2 44–2 56	20 3 2 0	20 4 (22 1)	T_R 4 30 4 85	20 5	T_R 9 5–11 0 22 6
			—	1 5	0 2	8 3	5 6	0 6	0 4	5 8	39 7	12 6	0 8	0 1	—	0 3	0 1	—	—	3 5[a]
			—	0 3	0	3 2	4	0 3	0 2	2 2	6 0	3 0	0 4	0 3	—	0 3	0 3	—	—	4 9[b]
O N	♀/32	70	—	Tr	—	16 4	3 3	—	—	4 9	34 2	19 7	1 2	Tr	1 7	1 0	—	—	—	17 5
		114	—	0 8	0 3	17 6	7 7	—	Tr	6 4	25 5	22 4	2 8	Tr	2 0	1 8	—	—	—	12 7
		120	—	2 2	0 6	20 7	11 3	—	Tr	5 2	28 9	26 2	3 2	Tr	—	—	1 5	—	—	—
K L	♀/48	125	—	1 4	Tr	26 0	6 4	—	—	9 0	40 8	14 8	1 5	—	—	—	Tr	—	—	Tr
		102	—	4 4	Tr	31 9	6 7	—	—	7 4	31 4	9 5	0 9	Tr	2 7	—	—	—	—	15 0
		64	—	1 5	Tr	22 3	6 6	—	—	6 1	37 6	15 0	1 9	—	—	—	Tr	Tr	—	9 0
		105	—	1 1	Tr	31 3	—	—	—	9 0	44 3	12 8	1 4	—	—	—	—	—	—	Tr
		44	—	0 7	Tr	30 9	—	—	—	6 7	40 2	15 5	0 5	0 9	—	—	—	—	—	4 6
H T	♀/63	53	—	0 9	0 3	0 7	7 4	—	Tr	8 5	33 5	22 0	2 4	Tr	Tr	—	0 7	—	—	3 5
		31	—	1 9	0 3	22 9	6 2	—	—	5 7	41 0	8 8	0 8	—	—	—	—	—	—	12 4
		60	—	1 1	Tr	19 5	11 6	—	—	4 4	38 6	13 5	0 9	—	Tr	—	—	Tr	—	10 3
		104	—	Tr	Tr	23 2	5 9	—	—	6 1	47 0	12 2	Tr	Tr	Tr	—	—	—	—	5 4
K Γ	♂/55	165	—	1 7	Tr	26 2	10 5	—	Tr	6 0	40 4	13 8	1 5	Tr	—	—	—	—	—	—
		105	—	1 0	0 4	27 0	9 7	—	—	4 6	40 9	14 0	1 3	Tr	Tr	—	1 0	—	—	Tr
S B	♀/59	103	—	2 1	0 5	22 2	5 3	—	—	5 7	37 4	14 2	1 7	—	—	—	—	1 1	—	9 4
		126	Tr	Tr	—	29 0	—	—	—	5 5	48 5	15 8	1 2	—	—	—	—	—	—	Tr
E B	♀/68	1 6	—	Tr	Tr	28 3	—	—	—	3 4	47 6	19 4	1 2	—	—	—	—	—	—	—
		121	—	1 7	0 2	26 1	9 0	—	—	6 9	39 8	14 4	1 8	—	—	—	Tr	—	—	—
K A	♂/32	214	1 5	4 1	1 5	14 0	8 5	5 3	—	9 9	35 3	10 6	1 3	Tr	1 0	—	0 8	—	—	6 ⌄
		163	—	3 7	0 8	26 0	—	—	2 0	9 6	28 1	9 8	—	0 7	1 0	—	—	—	—	17 4
		210	—	Tr	Tr	28 9	3 9	—	—	9 3	41 0	12 8	Tr	1 5	—	1 3	1 2	—	—	Tr

Abbreviations se Table I
[a] Normal value 13 women and 19 men
[b] Standard deviation

Table III *Phospholipids*

Value in mg per 100 ml of plasma if not otherwise indicated

Subject	Sex/Age	Phospho lipids	Fatty acid composition value in per cent of total																	
			1 0	14 0	14 1	16 0	16 1	17 0	17 1	18 0	18 1	18 2	18 3	0 0	0 3 2 0	0 4 (? 1)	T_R 4 30– 4 85	0 5	T_R 6 5– 7 0	T_R 9 5 11 0 / 22 6
			—	0 ?	0 1	30 0	0 8	0 4	0 ?	17 2	15 4	0 9	0 5	Tr	2 0	8 1	—	—	—	3 0[a]
			—	0 1	0 1	4 ?	0 8	0 2	0 5	9	7	3 9	0 3	—	1 0	1 9	—	—	—	? 1[b]
O N	/32	308	—	Tr	Tr	8 1	—	—	—	16 6	15 5	6 5	0 5	Tr	3	9 5	—	—	—	—
		?85	—	—	—	14 1	Tr	Tr	—	14 ?	17 5	?1 1	1 0	0 4	5 1	16 9	—	Tr	9	6 4
		244	—	0 2	0 4	17 0	?0	1 1	—	14 4	15 8	21	0 5	Tr	5 3	13 5	—	—	3 ?	4 8
K I	+/48	343	—	—	—	23 5	—	Tr	—	21 8	?0 5	24 9	Tr	—	2 2	7 1	—	—	—	—
		3 3	—	—	—	21 3	Tr	Tr	—	18 0	0 2	?5 0	0 5	Tr	1 1	9 6	—	—	—	2 2
		326	—	—	Tr	5 1	Tr	Tr	—	19 0	17 6	.3 1	Tr	Tr	? 1	8 0	—	1 1	—	4 0
		321	—	Tr	Tr	0 5	—	—	—	1) 1	25 9	2 8	Tr	—	1 1	7 1	1 1	—	—	2 2
		306	—	—	—	10 4	—	—	—	18 7	1) 1	4 2	—	Tr	2 0	5 6	—	—	—	—
H T	♀/63	334	—	0 3	0 7	0 4	2 3	Tr	—	16 6	18 1	2?9	0 6	Tr	2 ?	7 5	—	Tr	1 4	4 0
		194	—	0 4	0 5	2) 1	—	—	—	17 0	?0 5	2?)	Tr	—	3 6	6 0	—	—	—	Tr
		?11	—	Tr	Tr	35 2	—	—	—	14 3	17 9	21)	—	Tr	1 8	6 7	—	—	—	2 1
		188	—	Tr	Tr	24 0	—	—	—	16 3	18 4	?1 6	Tr	Tr	3 1	) 8	—	1 ?	1 ?	4 4
K I	♂/55	443	—	Tr	Tr	6 2	—	Tr	Tr	0 2	19 5	23 6	Tr	Tr	0 7	9 8	—	—	—	Tr
		219	—	Tr	0 4	24 2	Tr	—	—	15 3	16 3	18 3	0 4	Tr	6 2	13 4	—	1 3	1 6	? 5
S B	/5)	44)	—	Tr	Tr	?5 8	—	Tr	—	1) 6	16 6	?5 1	Tr	Tr	1 2	8 3	Tr	—	—	3 3
		251	—	—	—	24 4	—	Tr	—	18 5	17	5 7	Tr	—	2 8	11 2	Tr	—	—	—
I B	/(8	259	Tr	Tr	Tr	25	—	–	–	15 7	0 8	21)	—	Tr	1 5	8 3	1 2	—	—	5 ?
		?61	–	Tr	–	0 1	Tr	–	—	?1 3	16 6	5 1	—	Tr	Tr	10 8	1)	—	—	4 1
K A	♀/?	300	—	Tr	Tr	21 6		Tr	–	18 ?	18 0	21 7	Tr	Tr	2 ?	10 7	—	—	5)	1 7
		215	–	Tr	Tr	21 8	—	–	–	17 0	17 4	2? 4	—	0 6	1 8	11 0	Tr	—	3 1	2 7
		217	–		—	6 7	—	Tr	Tr	18 3	16 2	21 1		0 6	1 4	11 1	—	—	—	4 (

Abbreviations see Table I
[a] Normal value 13 women and 0 men
[b] Standard deviation

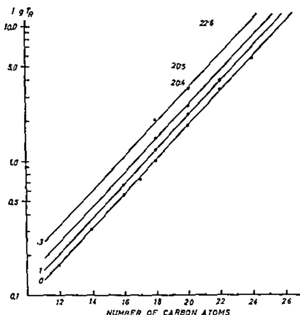


Fig 1 Logarithm of retention value (T_R) of methylated fatty acids plotted against the number of carbon atoms in the molecule obtained in assays with pure substances

benzene Benzene-diethyl ether 9 1 was used as a solvent The chromatography was carried out by the sandwich technique Visualisation was achieved by spraying at the borders with 2 7-dichlor fluorescin 0 2 w/v in 96% ethyl alcohol The bands were then scraped off and re extracted with diethyl ether and gas liquid chromato graphy was repeated

RESULTS

he results are given in Tables I–III

In all the hypothyroid patients moderate to marked *hypercholesterolemia* was observed After treatment the plasma cholesterol level decreased The esterification rate of cholesterol was within the normal range in the hypothyroid patients and no significant changes were noted during correc tion of the hypothyroid state These results are in accordance with the findings of Furman et al (11)

All patients except one a diabetic had *tri glyceride* values within the normal range and no changes were observed when treatment was started

The concentration of the *phospholipids* showed a nearly uniform decrease when the hypothyroid patients were treated

In the *plasma lipoprotein pattern* by far the most common abnormality was an increase of the β lipoproteins which according to Fredrickson s system indicates type II hyperlipoproteinemia After treatment these alterations disappeared One patient the diabetic (K A) did not belong to this type His lipoprotein pattern seemed to be long to the combined type V hyperlipoproteinemia with a slight chylomicronemia as well as increased pre β lipoproteins

No changes seemed to occur in the α lipopro teins

Fatty acid pattern In the fatty acid composi tion of the hypothyroid patients three types of abnormalities were observed namely an abnormal accumulation of a component with a very high retention value (T_R) the occurrence of components with retention values not found in the 31 normal persons investigated and a relatively increased amount of arachidonic acid (C20 4) after the hyperthyroid state had been corrected

The three abnormalities were not seen uniformly in all patients but formed a common pattern

The first abnormality the accumulation of a component with a very high retention value (9 5–11 0), occurred in the cholesterol esters sometimes in a very high degree and to a lesser degree in the triglycerides but not in the phospholipids From several assays it was known that a fatty acid with a retention value around 10 is an indication of the fatty acid C22 6 Δ4 7 10 13 16 19 which normally appears in relatively small amounts in human plasma (where it can be synthetized from linolenic acid) However as seen in Fig 1 the saturated fatty acid C26 0 also satisfies the reten tion value obtained Normally C26 0 is *not* present in human plasma (22)

To identify this fatty acid a thin layer chromato graphy on $AgNO_3$ enriched silica gel layer was performed Good separation according to the con tent of double bonds was obtained and in each case of increased concentration of the unidentified component it could be isolated from the *saturated* fatty acid group which moves in the solvent front of the chromatogram This strongly supports the idea of a long chain saturated fatty acid viz C26 0 An example is shown in Fig 2 However combination with a lesser amount of C22 6 cannot be excluded

The second abnormality in the hypothyroid pa tients was the frequent occurrence of components with retention values not found in the normal sub jects investigated The components were present in all three lipid fractions and may be arranged in *four groups The first group* contained fatty acid(s) with retention values rather close to 2 50

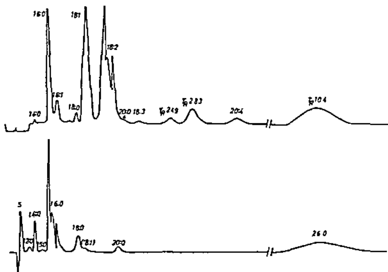


Fig. Gas-liquid chromatogram of the methylated fatty acids from the cholesterol esters (patient O. N.) before and after isolation of the saturated fraction by thin layer chromatography. Except for a trace of C18:1 only saturated components are represented in the second curve. *S* means solvent.

The component occurred rather often but frequently in too small amounts for detection by gas liquid chromatography after attempts to isolate it by thin layer chromatography. Where this could be achieved the component was isolated from the saturated group. From Fig. 1 the only possibility then seems to be C21:0.

The second group of fatty acids with retention values about 2.88 occurred twice (O. N. and K. L.) in the cholesterol esters in amounts which it was hoped would enable identification by gas liquid chromatography after separation by thin layer chromatography. Identification which satisfied our standard curve (Fig. 1) was achieved in only one of the patients where the component was isolated among the monoenes. This seems to confirm the occurrence of the fatty acid C21:1.

The third group of fatty acids with retention values of 4.30–4.85 did not occur in amounts big enough for identification. C22:2 and C23:0 are possibilities according to Fig. 1. This group is probably rather inhomogeneous.

The fourth group with the retention values of 6.5–7.0 was seen rather often and occurred twice (O. N. and K. A.) in sufficient amounts for identification by thin layer chromatography. It was found among the components with one double bond, making C24:1 very probable.

The third abnormality of the fatty acid pattern was the relative increase in arachidonic acid after treatment of the hypothyrosis. In the cholesterol esters as well as in the phospholipids it occurred in five out of the six patients who were followed to the euthyroid state.

The long-chain saturated fatty acid C22:0 could also be detected in our patients after its separation from C20:3 by thin layer chromatography. Our impression is that C22:0 does not occur in euthyroid patients on a normal diet but confirmatory results have not been obtained yet.

When the hypothyroidism was treated the above mentioned abnormalities disappeared on the whole. The changes in the phospholipids seemed slightly more resistant than those of the other lipid fractions.

DISCUSSION

No attempt was made to carry out a quantitative lipoprotein estimation. Paper lipoprotein electrophoresis was only used as a qualitative measure of the type of hyperlipoproteinemia. In trying to ascertain whether the decrease in the plasma phospholipids was due solely to the obvious decrease in the β lipoproteins or might also be due to a decrease in the phospholipid rich α lipoproteins (high density lipoproteins) a semi-quantitative estimation of this fraction was made from the strips.

Our impression is that the α lipoproteins do not undergo any great changes when the hypothyroid state is corrected. Opinions differ on this subject in the literature (3, 11, 18).

As mentioned earlier each of the three major

lipid groups in human plasma has its own characteristic fatty acid pattern which is relatively stable to dietary fatty loads The human organism is capable of synthetizing saturated fatty acids de novo from acetyl CoA malonyl CoA and NADPH and to elongate and desaturate intrinsic and extrinsic fatty acids by adding two carbon groups at a time (Acetyl CoA) or substrating two hydrogen atoms

These po sibilities are not unlimit d The de novo synthesis is limited to palmitic acid and stearic acid whereas by elongation saturated fatty acids of a higher order can be formed The desaturation system is not able to introduce a double bond beyond the 9th carbon atom in the molecule Therefore unsaturated fatty acids with this property have to be added to the mammalian organism for synthesis of polyunsaturated fatty acids of higher order (7 22)

The higher polyunsaturated fatty acids have a marked lowering effect on serum cholesterol This effect is the greater the longer the chain and the larger the number of double bonds (21) Linoleic acid seems only to be active as a precursor of arachidonic acid (7) and the most active is C22 6 Δ4 7 10 13 16 19 The long-chain saturated fatty acids seem to have the opposite effect that of raising the serum cholesterol level

The possible effect of the different fatty acids on the other plasma lipid groups does not appear to be at all clear

The mechanism of the fatty acids with regard to lipid metabolism is not fully understood One of the major problems is whether free or esterified cholesterol is the form in which it is oxidized The first step in the conversion of cholesterol to bile acid is a hydroxylation of the steroid in the 7th position (2) By folding the molecule a polyunsaturated fatty acid in cis form in cholesterol ester is able to bring its second double bond close to the carbon 7 of cholesterol

Although it is well established that feeding polyunsaturated dietary fats lowers the serum cholesterol concentration attempts to ascertain if under these circumstances an increase in the production of bile acid takes place have not given uniform results (16) In hypothyroid patients decreased formation of bile acid has been observed which in turn increased when the patients were treated (2)

It was thought that a possible explanation of the hyperlipidemia in hypothyrosis could be that lack of thyroid hormone changes the fatty acid synthesis and the rebuilding from the dietary fatty acids in such a way that the esters subsequently formed are of a type difficult for the human organism to metabolize

The results obtained in this study app ar to support this idea as we found abnormal amounts of what seemed to be long chain saturated fatty acids possibly with a carbon number up to twenty six Long chain monoenes and maybe dienes of a type not seen in normal subjects were also found Some of the results seem to indicate the possibility of the occurrence of long chain fatty acids with an uneven carbon number in the molecule

A possible explanation of our findings is that the desaturation system depends more on the thyroid hormone than does the elongation system When the saturated fatty acids are not desaturated their accumulation could possibly give rise to further elongation and result in a synthesis of long chain fatty acids not seen in euthyroid subjects

CONCLUSION

The results are presented of a detailed investigation of the plasma lipids in seven patients with hypothyrosis

The hypothesis seems to be confirmed that one of the causes of hyperlipidemia in hypothyrosis could be that thyroid hormone deficiency qualitatively changes the fatty acid synthesis and thus causes the formation of esters not easily metabolized by the organism as the normal esters

This problem requires further investigation more particularly a better identification of the abnormal fatty acids found in order to get a more precise picture of where the thyroid hormone acts in lipid metabolism

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REFERENCES

1 Ahrens E Jr Insull W., Jr Hirsch J Stoffel W Peterson M Farquhar J Miller T & Thomasson, H The effect on human serum lipids of a dietary fat highly unsaturated but poor in essential fatty acids Lancet 1 115 1959

B rgstrom, S., Danielsen, H & Samuelsen B Formation and metabolism of bile acids. In Lipide metabolism (ed K. Bloch) p 291 John Wiley & Sons New York and London 1960

3 Cornwell D G Kru er F A Hamwi, G J & Brown, J B Studies on the characterization of human serum lipoproteins separated by ultracentrifugation in a density grad ent Amer J Clin Nutr 9 24 1961

4 Dole V., James, A Webb J Rizack, M & Sturman, M The fatty acid patterns of plasma lipids durin alimentary lipemia. J Clin. Invest 38 1544 1959

5 Dyerberg J., Hauerbach T Holm Andersen, U Holme N., Kruse F & Nielsen I A Uptake of =J labelled l triiodothyronine by human erythrocytes and Sephadex G*5 medium as an in vitro test of thyreoid function Nord Med 77 43 1967

6 Eg stein, M & Kreutz, F H Eine neu Bestimmun der Neutralfette im Blutserum und Gewebe Klin Wschr 44 262, 1966

7 Elovson J Biosynthesis and distribution of the polyunsaturated fatty acid In Polyunsaturated fatty acids as nutrients (ed G Blix) p 23 Almqvist & Wiksell Uppsala 1966

8 Folch, J Le s M & Sloane Stanley G H A simple method for the isolation and purification of total lipides from animal tissues J Biol Chem. . 6 497 1957

9 Fredrickson M D Levy R. I & Lees R S Fat transport in lipoproteins — An integrated approach to mechanisms and disorders New Engl J Med 276 34 94 148 215 273 1967

10 Freeman N K Lindgren F T & N chols A V The chemistry of serum l poproteins In Progress in the chemistry of fats and other lipids (ed R T Holman, W O Lundberg & T Malkin) vol 6 p 215 Macmillan, New York 1963

11 Furman, R. H Howard R. P Lakshmi K & Norcia L N The serum lipids and lipoproteins in normal and hyperlipidem c subjects as determined by preparative ultracentrifugation Amer J Clin. Nutr 9 73 1961

12 Jencks W P & Durrum E L Paper electrophoresis as a quantitative method The staining of serum lipoproteins J Clin Invest 39 1437 1955

13 Jest ng, E. & Bang, H O Gas liquid chromatography applied as a control for methylation processes Dan Med Bull 8 169 1961

14 Lees R. S & Hatch, F T Sharper separation of lipoprote n species by paper electrophoresis in albumin-containing buffer J Lab Cl n Med 61 518 1963

15 Lindgren, F T Nichols, A V & Wills R D Fatty acid distributions in serum lipids and serum lipoproteins Amer J Clin Nutr 9 13 1961

16 Lindstedt S The effect of unsaturated fatty acids on cholesterol metabol sm In Polyunsaturated fatty acids as nutrients (ed G Blix) p 65 Almqvist & Wiksell Uppsala 1966

17 Morris L J Separation of higher fatty acid isomers and vinylogues by thin layer chromatography Chem and Ind (London) 27 1238 1962

18 O Hara, D D Porte D Jr & Williams R. H The effect of diet and thyroxin on plasma lipids in myxedema Metabolism 15 123 1966

19 Olivecrona T The metabolism of 1 C palmitic acid in the rat Acta physiol scand 54 295 1962

20 Runde I A standardized direct method for total cholesterol determination in serum with a combined reagent Scand J clin. Lab Invest. 18 461 1966

21 Sinclair H M The importance of structure in dietary fatty acids In Metabolism and physiological significance of lipids (ed R. M C Dawson & D N Rhodes) p 625 John Wil y & Sons London New York, Sidney 1964

22 Wakil S J The synthesis of fatty acids in animal tissues In Metabolism and physiological significance of lipids (ed R. M C Dawson & D N Rhodes) p 3 John Wiley & Sons London New York Sidney 1964

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PREDNISONE GLUCOSE TOLERANCE AND SERUM LIPIDS IN SURVIVORS OF MYOCARDIAL INFARCTION

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Abstract Glucose tolerance has been examined by means of a prednisone glucose tolerance (PGT) test in 41 patients with clinically documented myocardial infarction 3 to 4 weeks after the attack and in ag matched control subjects without clinical evidence of cardiovascular disease In addition serum cholesterol and triglyceride levels have been determined and free fatty acid (FFA) concentrations measured prior to the PGT test and after administration of the glucose load

Abnormal glucose tolerance curves were obtained in 53 6% of the patients 3 to 4 weeks after the infarction and in 25 8 of the controls Seventeen patients were retested approximately six months after the first test and glucose tolerance in seven patients with initially abnormal PGT curves was found to be within normal limits while further impairment was observed in only four cases A significant correlation between the PGT tests and serum cholesterol levels was found to exist only in patients over the age of 60 while no correlation could be observed between prednisone glucose tolerance and serum triglycerides or fasting levels of FFA The mean decrease of FFA 1 hour after the administration of glucose was s ghtly less in the pat ents with myocardial infarction than in the controls, while a decrease of FFA to levels below 00 μEq/l was observed in approximately one third of the patients 1 and/or 3 hours after the glucose load

It is concluded that the impairment of glucose tolerance which frequently can be observed after recent myocardial infarction is probably due only in a minority of cases to a latent diabetic condition and that other factors which are known to influence carbohydrate metabolism must be taken into consideration in explaining the observed disturbances of glucose homeostasis

The association of myocardial infarction with manifest diabetes mellitus is well known and so is the usually transient glucosuria which may occur during the acute stage of infarction Studies concerning oral glucose tolerance (1 3 4 5 7 12 17 20 21 25 32) or intravenous glucose tolerance (17 22 33) made during recent years in patients with coronary heart disease have shown a surprisingly high incidence of impairment of glucose tolerance after recent myocardial infarction and have given rise to discussion concerning the possible significance of these abnormalities of carbohydrate metabolism in the pathogenesis of coronary atherosclerosis

There seems however to be no agreement about the persistence of the observed impairment of glucose tolerance which is associated with myocardial infarction A decrease in the incidence of abnormal glucose tolerance following a more prolonged interval after the acute stage of infarction has thus been observed during the course of some studies (7 15 25) while other investigators have expressed the view that the duration between infarction and testing does not significantly influence the results of the glucose tolerance tests (22 33) Few studies on the other hand have included data which would allow conclusions to be drawn about the predictive value of glucose tolerance tests concerning the development of clinical diabetes in patients with coronary heart disease

Steroid modified glucose tolerance tests which were first introduced by Fajans and Conn in 1954 (11) are regarded as a more sensitive means than unmodified glucose tolerance tests in detecting early abnormalities of glucose tolerance (6) and might therefore be expected to offer additional information concerning the incidence and nature of the early disturbances of carbohydrate metabolism in patients with myocardial infarction Since only a few studies concerning steroid modified glucose tolerance in patients with coronary heart disease have been published to date prednisone glucose tolerance (PGT) tests were performed in a group of hospitalized patients

Table I Number of positive PGT tests in different age groups of patients with myocardial infarction and in control subjects

	Myocardial infarction			Control group		
Age groups	No of subjects	Positive PGT tests	Negative PGT tests	No of subjects	Positive PGT tests	Negative PGT tests
< 50	18	7	11	13	4	9
50–59	15	8	7	9	2	7
60–69	8	7	1	9	"	7
Total	41 (100)	22 (53 6)	19 (46 4)	31 (100)	8 (25 8)	23 (74 2)

with recent myocardial infarction 3 to 4 weeks after the attack and repeated approximately six months after the initial test Similar tests were performed in age matched control subjects without clinical evidence of cardiovascular disease In addition serum cholesterol and triglyceride levels were determined and free fatty acids (FFA) measured during the PGT tests

MATERIAL AND METHODS

The study was performed in 41 patients with myocardial infarction (39 males and two females) who were hospitalized at the Department of Medicine of the Maria Hospital or at the IV Department of Medicine of the University of Helsinki A complete evaluation including serial ECGs and determinations of SGOT was performed in all patients and only cases with clinically documented myocardial infarction were included The average age of he patients was 57 4 years (range 36 to 69 y) and that of a control material consisting of 31 healthy volunteers or patients admitted to a medical ward for the evaluation of minor non vascular illnesses was 54 1 years (range 40 to 69 y) There were no significant differences in body weight between patients and controls Subjects with obesity of 0 or more above the ideal weight were not included and individuals with previously known disturbances of carbohydrate metabolism or a family history of diabetes were likewise excluded from the study

Glucose tolerance was determined after two or three days on a diet rich in carbohydrate by means of a PGT test which was performed in the patients with myocardial infarction during the stage of convalescence 3 to 4 weeks after the attack In 17 patients retests were performed 4 to 10 months (average six months) after the initial test The tests were performed according to the original cortisone glucose tolerance test described by Fajans and Conn (11) substituting 10 mg of prednisone for each 50 mg of cortisone Persons weighing more than 7_5 kg received 12 5 mg of prednisone 8 / hours and again 2 hours before a standard glucose tolerance test, which was performed by administering to the test subjects 1 g of glucose per kg body weight Capillary blood was withdrawn for the blood glucose determinations at 0 ½ 1 1½, 2 and 3 hours after the administration of the glucose load and examined by a method for true blood glucose estimation (14)

A PGT test was regarded as positive according to the criteria for normality suggested by Conn and Fajans (6) if blood glucose values in excess of 160 mg% at 1 hour *and* blood glucose values in excess of 140 mg mg at 2 hours were obtained In order to facilitate comparison of the results of the PGT tests with serum lipid levels each glucose tolerance test was also expressed as an Area Index an approximation of the area under the PGT curve which according to Vecchio et al (31) was calculated from the formula

$$\frac{A}{2}+B+C+D+\frac{E}{2}$$

where A is the fasting blood glucose value and B C D and E the subsequent values for the next two hours taken at half hour intervals In this way a single numerical value is obtained to express the entire tolerance test, providing an index for the blood glucose level for the entire duration of the test

Prior to the administration of the glucose load blood samples were obtained for th determination of serum lipids Serum cholesterol was determined by the method of Pearson et al (19) serum triglycerides by a slightly modified procedure of Van Handel and Zilversmit (30) and FFA levels by the Dole procedure as modified by Trout et al (27) Blood samples for the determination of FFA were also obtained 1 hour and 3 hours after the glucose load simultaneously with the corresponding blood samples for the determination of blood glucose (The analyses of serum triglycerides and the determinations of free fatty acids were carried out at the Lipid Research Laboratory of the Third Department of Medicine University of Helsinki)

RESULTS

Table I shows the number of positive and negative PGT tests obtained in patients with myocardial infarction 3 to 4 weeks after the atta k

and the re ults of similar tests obtained in age matched controls The material has been divided into three age groups according to decades Posi tive PGT tests were in all three groups observed more frequently in the survivors of myocardial infarction than in the controls and an increase in the proportion of patients who had abnormal tests was seen with increasing age

The results of the PGT tests obtained in the same group of patients and control subjects have in Fig 1 been expressed as the mean PGT Area Indexes of the respective age groups In the pa tients with myo ardial infarction in all three groups the mean PGT Area Indexes were found to be higher than although not statistically dif ferent from the Area Indexes obtained in the respe tive control groups The impairment of glucose tolerance was however found to be statistically significant ($P<0.02$) when the three age groups were combined and the PGT Area Indexes obtained in all patients with myocardial infarction were compared with the corresponding indexes obtained in the control material

The results of retests performed in 17 patients with myocardial infarction 4 to 10 months after the initial PGT test are shown in Fig 2 It can

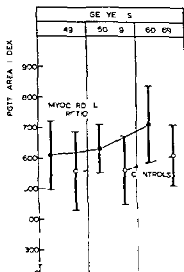


Fig 1 M an PGT test Area Indexes and standard devia tions in three age groups of patients with myocardial infarction and in age matched controls The PGT test Area Index s of the patients with myocardial infarction ha e been calculated from glucose tolerance tests per formed three to four weeks after the acute attack

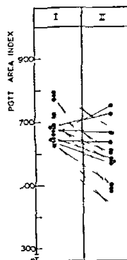


Fig 2 PGT test Area Indexes calculated from glucose tolerance tests performed in patients with myocardial in farction 3 to 4 weeks after the acute attack (I) and approximately six months later (II)

be seen that at this time the PGT Area Indexes were in most cases lower than during the initial test performed in the same patients 3 to 4 weeks after the infarction In seven patients originally abnormal PGT tests had at the time of retesting reverted to normal while some improvement of glucose tolerance was observed in six additional cases and PGT curves were found to be impaired in only four patients There was no significant change in body weight of the patients at the time of retesting

Mean levels and standard deviations of serum cholesterol and triglycerides in three age groups of patients 3 to 4 weeks after myocardial infarc tion are shown in Table II Mean serum cho- lesterol values were found to be similar in all

Table II *Mean levels and standard deviations of serum cholesterol and triglycerides in patients with myocardial infarction*

	< 50	50–59	60–69 y
Cholesterol (m)			
n	18	15	8
Mean	253	.66	63
S.D	58 ?	69 6	58
Triglycerides (mg)			
n	16	15	6
Mean	114	130	152
S D	43 9	51 3	59 1

Table III *FFA levels before and after the oral administration of glucose in patients with myocardial infarction and in control subjects*

	Mean age	FFA (µEq/l)		
		0 h	1 h	3 h
Myocardial infarction	61 3			
n		25	25	25
Mean		642 3	397 (−38 2)[a]	300 (−53 3)[a]
S D		263 3	159 8	143 3
Control subjects	67 3			
n		2?	21	?2
Mean		634	374 (−41)[a]	3 7 (−48 4)
S D		157 7	158	242

[a] Percent decrease of initial FFA levels after the administration of the glucose load

Table IV *Distribution of positive and negative PGT tests in patients with myocardial infarction in relation to the amount of FFA decrease observed after the administration of glucose*

PGT test	FFA levels 1 to 3 h after the oral administration of glucose: > 200 µEq/l	< 200 µEq/l	Total
Positive	14	8	22
Negative	13	5	18
Total	27	13	40

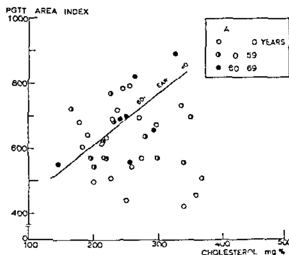


Fig 3 Relation of PGT test Area Indexes to serum cholesterol levels in patients with myocardial infarction

three age groups while serum triglyceride levels showed a tendency to increase with increasing

The relation between serum cholesterol and PGT tests performed in patients with myocardial infarction 3 to 4 weeks after the attack can be seen in Fig 3 A statistically significant correlation ($P < 0\ 05$) between serum cholesterol levels and the PGT Area Indexes was observed only in the patients over the age of 60 while no correlation was found to exist between serum triglycerides and prednisone glucose tolerance

Table III shows mean FFA levels before and after the administration of glucose to patients with recent myocardial infarction and the results of similar tests performed in control subjects Mean fasting levels of FFA were found to be similar in patients and controls and no significant difference could be observed between the two groups in decrease of FFA after administration of the glucose load

The number of patients with myocardial infarction in whom the FFA level after administration of the glucose load was less than 200 µEq/l is seen in Table IV A decrease of FFA values below this level 1 and/or 3 hours after the glucose load was observed in 13 out of a total of 40 patients Of these 13 patients eight had a positive PGT test while the distribution of positive and negative tests was found to be similar in patients in whom FFA levels after the administration of glucose did not decrease below 200 µEq/l

There was no correlation between the results of the PGT tests and fasting levels of FFA

DISCUSSION

Prednisone glucose tolerance was found in the present study to be significantly impaired in patients with myocardial infarction 3 to 4 weeks

after the attack The number of abnormal PGT tests was however not large compared to the number of abnormal curves obtained in patients with recent myocardial infarction after unmodified oral glucose tolerance tests which has been found to range from 41 up to 85 % (4 5 20 25 32) Reaven et al (21) on the other hand following the administration of cortisone observed only insignificant differences in glucose tolerance between patients with myocardial infarction and age matched control subjects Steroid modified glucose tolerance tests would therefore not seem to be more sensitive than regular tests in detecting early disturbances of carbohydrate metabolism in patients with coronary heart disease

The results obtained by different workers are however not directly comparable because the glucose tolerance tests have in many instances been carried out at different intervals after the infarction The effect of ageing on glucose tolerance which in the present study was reflected by the greater number of abnormal PGT tests in patients with myocardial infarction with increasing age must moreover be taken into consideration in comparing glucose tolerance in survivors of myocardial infarction belonging to different age groups In some studies (1 32) on the other hand control subjects have consisted of comparatively young healthy individuals in whom impairment of glucose tolerance is known to occur less frequently than in subjects belonging to the age groups in which coronary heart disease is prevalent

During the course of the present study glucose tolerance was found to be improved in most cases subjected to follow up studies This is in accordance with the results of Sowton (25) who made follow up studies of 30 patients with myocardial infarction and found abnormal oral glucose tolerance curves six months after the acute episode to be diminished from 73 to 43 while three years later only 27 of 15 patients had abnormal glucose tolerance Similar observations have been made by Kimber (15) and by Datey et al (7) who found that the incidence of hyperglycemia in 57 cases of myocardial infarction was 70 % at the onset of the attack while abnormal glucose tolerance was found to be transient in 38 % of the cases In contrast to the above mentioned reports Wahlberg (33) in his study of intravenous glucose tolerance in patients with myocardial infarction observed only an insignificant increase of the mean k values in patients who were retested within six months or 7 to 24 months after the infarction while an insignificant decrease of the mean k values was observed in patients retested more than 24 months later

Only few studies have dealt with the predictive value of glucose tolerance tests concerning the development of clinical diabetes in patients with myocardial infarction (7 10 25 26) The incidence of diabetes three years after the acute episode was in the series of Sowton (25) 13 while Datey et al (7) similarly observed that clinical diabetes had developed in approximately 14 % of their patients who had diabetic glucose tolerance curves shortly after the infarction Eckerstrom (10) on the other hand found that 12 patients with initial hyperglycemia had normal glucose tolerance curves ten years after the acute attack

It would thus seem probable that abnormalities of glucose tolerance after recent myocardial infarction are due only in a minority of patients to a latent diabetic condition and that other factors which are known to influence carbohydrate metabolism such as derangements of hypothalamic function caused by circulatory disturbances of the brain (20) increased secretion of adrenocortical steroids or an increased release of adrenaline (8) liver cell necrosis due to shock (25) low-carbohydrate diets and prolonged bed rest must also be taken into consideration in explaining the observed disturbances of glucose homeostasis

The correlation between prednisone glucose tolerance and serum cholesterol or triglyceride concentrations was in the present study found to be poor except for the positive correlation between the PGT tests and serum cholesterol observed in the small group of patients over the age of 60 A lack of correlation between circulating levels of these serum lipids and glucose tolerance has similarly been observed in previous studies (1 21 25 33) and these observations together with the absence of correlation between serum immunoreactive insulin on the one hand and disturbances of carbohydrate or lipid metabolism on the other (17) would seem to oppose the view that the observed deviations from normal in the carbohydrate metabolism of pa

tients with myocardial infarction may play a major role in the pathogenesis of the coronary artery disease

Mean fasting levels of FFA 3 to 4 weeks after myocardial infarction were likewise not found in the present study to be different from normal Other investigators have observed high FFA levels immediately after infarction (16) while Rifkind (22) in agreement with the present study found that FFA levels were not different from controls 3 weeks after infarction although the values were found to be high in patients with a more distant infarction These differences in FFA levels measured at different points of time after the infarction are however not surprising since many factors including diet physical activity smoking and emotional stress are known to influence FFA concentrations

The mean response of FFA levels to glucose which is usually less pronounced in diabetics than in normal subjects (2 9 13 23) was during the present study found to be only slightly less pronounced in patients with myocardial infarction than in control subjects Although a sluggish response of FFA to the oral administration of glucose has been observed by some workers in patients convalescing from myocardial infarction (24) Tzagournis et al as in the present study recently found that the FFA response to glucose in young patients with coronary heart disease was virtually identical to that of normal subjects (28) while Cohen and Shafrir (5) observed in two thirds of their 43 patients three weeks after myocardial infarction a prompt and long lasting decrease of FFA after an oral glucose tolerance test The adequate response of FFA to glucose which was observed in several cases in the presence of impaired glucose tolerance was attributed by these workers to a differential effectiveness of insulin action in adipose and non adipose tissue which according to Vallance-Owen and Ashton (29) could be explained by the appearance of insulin antagonists in patients with myocardial infarction

It may be concluded that so far there is only little evidence that the often transient impairment of glucose tolerance which is frequently observed after recent myocardial infarction is correlated to alterations of lipid metabolism Long term follow up studies in patients with coronary heart disease in whom signs of deranged carbohydrate metabolism are detected would seem to offer a means of assessing the relative importance of different metabolic abnormalities as contributory factors in the pathogenesis of coronary atherosclerosis which according to current concepts (18 28) can best be explained by the interaction of various atherogenetic factors over a period of many years

REFERENCES

1 Aleksandrow D Ciświcka Sznajderman M Ignatowska H & Wocial B Studies on disturbances of carbohydrate metabolism in atherosclerosis J Atheroscler Res 2 171 1962

2 Bierman E L Dole V P & Roberts T N An abnormality of nonesterified fatty acid metabolism in diabetes mellitus Diabetes 6 475 1957

3 Buchele S Über die Beziehung zwischen Herzinfarkt und Diabetes mellitus unter besonderer Berucksichtigung des latenten Diabetes Schweiz med Wschr 97 74 1962

4 Bohle E & Schrade W Über latente Storungen des Kohlenhydratstoffwechsels bei nichtdiabetischen Arteriosklerotikern Munch med Wschr 102 565 1960

5 Cohen, A M & Shafrir E Carbohydrate metabolism in myocardial infarction Behavior of blood glucose and free fatty acids after glucose loading Diabetes 14 84 1965

6 Conn J W & Fajans S S The prediabetic state Amer J Med 31 839 1961

7 Datey K K & Nanda N C Hyperglycemia after acute myocardial infarction Its relation to diabetes mellitus New Engl J Med 276 262 1967

8 De Bodo R C & Altszuler N Hormonal regulation of glucose production and utilization In Clinical diabetes mellitus (ed M Ellenberg and H Rifkin) p 91 McGraw Hill New York 1967

9 Dole V P A relation between nonesterified fatty acid in human blood plasma and the metabolism of glucose J clin Invest 35 150 1956

10 Eckerstrom S Clinical and prognostic aspects of acute coronary occlusion Acta med scand Suppl 250 1951

11 Fajans S S & Conn, J W The approach for the prediction of diabetes mellitus by modification of the glucose tolerance test with cortisone Diabetes 3 96 1954

12 Frehner H U & Wegmann, T Zur Fra e der Hyperglykamie und Glukosurie beim frischen Herzinfarkt Schweiz. med. Wschr 93 1592 1963

13 Hales C N Walker J B., Garland P B & Randle P J Fasting plasma concentrations of insulin non esterified fatty acids glycerol and glucose in the early detection of diabetes mellitus Lancet 1 65 1965

14 Hyvarinen A & Nikkilä E A Specific determination of blood glucose with o-toluidine Clin chim Acta 7 140 1962

15 Kimber R. J Glucose tolerance after myocardial infarction Med. J Aust 4 687 1965
16 Kurien V A. & Oliver M F Serum free fatty acids after acute myocardial infarction and cerebral vascular occlusion. Lancet 2 12_ 1966
17 Nikkila, E A Miettinen T A Ves nne M R & Pelkonen R Plasma insulin in coronary heart disease Lancet ? 508 1965
18 Ostrander L D Jr., Neff B J Block W D Francis, T Jr & Epstein F H Hyperglycemia and hypertriglyceridemia among persons with coronary heart disease Ann intern. Med 67 34 1967
19 Pearson, S Stern S & McGavack T H Rapid accurate method for the determination of total cholesterol in serum Analyt Chem 25 813 1953
0 Raab A P & Rabinowitz, M A. Glycosuria and hyperglycemia in coronary thrombosis J Amer med Ass 106 1705 1936
1 Reaven, G., Calciano A Cody R Lucas, C & Miller R. Carbohydrate intolerance and hyperlipemia in patients with myocardial infarction without known diabetes mellitus J clin. Endocr 23 1013 1963
— Rifkind, B M Plasma free fatty acid levels and intra enous glucose tolerance after myocardial infarction J Atheroscler Res 6 26 1966
23 Shafrir E. & Gutman, A Patterns of decrease of free fatty ac ds durin glucose tolerance tests Diabetes 14 77 1965
?4 Soloff L. A & Schwartz, H Relationship between glucose and fatty acid in myocardial infarction Lancet 1 449 1966
25 Sowton E Cardiac infarction and the glucose tolerance test Brit med J 1 84 196_
?6 Spuhler O V Herzinfarkt, Pankrea und Kohlenhydratstoffwechselstorungen Schweiz med Wschr 73 1458 1943
27 Trout D L Estes E H Jr & Friedberg, S J Titration of free fatty acids of plasma A study of current methods and a new modification J Lipid Res 1 199 1960
_8 Tzagournı M Seidenst cker J F & Hamwi G J Serum insulin carbohydrate and lipid abnormalities in pat ents with premature coronary heart disease Ann. intern Med 67 42 1967
_9 Vallance Owen, J & Ashton, W L Cardiac infarction and insulin antagonism Lancet 1 1?26 1963
30 Van Handel N & Zil ersmit D B Micromethod for the direct determination of serum triglycerides J Lab clin Med. ?0 15_ 1957
31 Vecchio T J Oster H L & Smith D L Oral sodium tolbutamide and glucos tolerance tests Arch intern. Med 11? 161 196?
3? Waddell W R. & Field R. A Carbohydrate metabolism in atherosclerosis Metabolism 9 800 1960
33 Wahlberg, F Intravenous glucose tolerance in myocardial infarction, angina pectoris and intermittent claudication Acta med scand Suppl 4?3 1966

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THE DEVELOPMENT OF CELLULAR HYPERSENSITIVITY IN MAN AFTER A PRIMARY IMMUNIZATION

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Abstract The development of cellular and humoral hypersensitivity was followed during a primary immunization with brucella bacteria in 15 persons Both types of hypersensitivity were detected by in vitro techniques thereby enabling repeated recordings of the degree of hypersensitivity in each individual Cellular hypersensitivity as measured by the leucocyte migration test developed in two phases separated by a shorter period of non reactivity The first phase was present as early as two days after the immunization. Humoral hypersensitivity measured as agglutinating antibodies followed the usual pattern with rising titers after a latent period of 6–8 days Cellular hypersensitivity thus seemed to develop earlier than humoral hypersensitivity and apparently no correlation seemed to exist between these two parameters. The mechanism of the biphasic occurrence of cellular hypersensitivity especially the possible analogy with the so-called Jones-Mote s reactivity and the interrelationship between cellular and humoral hypersensitivity is discussed

The primary immune response of an organism is considered to develop in two directions namely a cellular hypersensitive state and/or a humoral hypersensitive state The distinction between these two hypersensitive states has been made mainly upon arbitrary criteria and has been of great value in defining and describing the characteristics of the two immunologic conditions Depending upon the immunization procedure the applied antigen and the immunized organism the hypersensitivity will develop in one of the two directions or both The question whether these two modes of immune reaction are unrelated or whether they represent the same basic immunologic process is still unsolved and subject to discussion

The study of the relationship between these two types of hypersensitivity would among other things demand an exact simultaneous recording of both types following a primary antigenic stimulus In order to avoid interference with the immunologic reaction to be investigated in vitro techniques must be considered most suitable as they allow multiple measurements in the same individual during the course of immunization

As to humoral hypersensitivity the technical basis of in vitro demonstration has been highly developed and the various steps of humoral antibody production are well clarified In contrast little is known about the sequential development of cellular hypersensitivity Some work has been done using the delayed intracutaneous reaction but this technique is not ideal because the antigen is introduced into the organism thereby altering the immunologic status to be examined

The development of an in vitro technique for detecting cellular hypersensitivity using peripheral human leucocytes (24) offers an opportunity of recording the different steps of cellular hypersensitivity following a primary immune stimulus

The purpose of the present study was to apply this technique together with the recording of humoral hypersensitivity in order to follow the development of both hypersensitive states during a primary immunization Brucella abortus Bang was selected as antigen because this bacterium is able to elicit both types of hypersensitivity through the same immunization procedure

MATERIAL AND METHODS

The material consisted of 15 persons with various internal medical diseases. The patients were all in a good general condition and did not receive cortisone therapy or other drugs supposed to interfere with the immunologic response

In order to ensure that the persons investigated indeed had a primary antigen stimulus, only brucella negative persons were examined The criterion for brucella nega

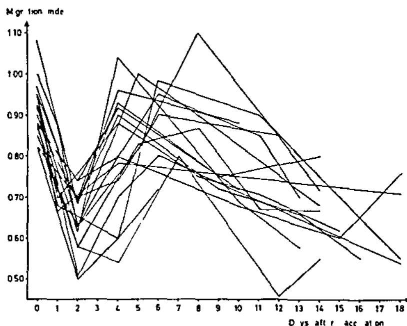


Fig 1 Variation of the migration indices in 15 persons after a primary immunization

tivity was a negative in vitro test of cellular hyper sensitivity using brucella bacteria as antig n and no demonstrable titers of brucella agglutinins in the serum (see below)

As primary immune stimulus 1000 mill Brucella abortus Bang bacteria were injected subcutaneousely in the thigh of each person

Determination of cellular hypersensitivity and humoral antibodies was done at the same time in each individual as described below All persons were investigated at time zero and subsequently every second and third day during the following 18 days

stration of cellular hypersensitivity

in vitro detection of cellular hypersensitivity was formed with peripheral human leucocytes according to the technique described in detail in a previous paper (?4) The principle of the method is based on the in hibitory effect of an anti en upon the migration of leucocytes in vitro in case the cells originate from an organism with cellular hypersensitivity to the same an tigen. The migration of the leucocytes is measured in the presence and absence of Brucella bacteria The action of antigen upon the cell migration is expressed in the so-called migration index M/M_o where M_o represents the average migration area of the antigen containing cultures and M the average migration area of the control cultures The migration index thus expresses the inhibi tion induced by the antigen in such a way that the more pronounced the inhibition the lower the migration index The Brucella bacteria were of the same kind as used in the immunization

The degree of migration inhibition has previously been shown to correlate with the degree of cellular hyper sensitivity as expressed by the delayed intracutaneous reaction (25) According to these experiments a migra tion index of 0 78 was the borderline between brucella positive and brucella negative obs rvations Consequently a migration index below 0 78 was the criterion of brucella hypersensitivity and persons were considered brucella negative if they had a migration index above 0 78

Demonstration of humoral hypersensiti ity

Circulating antibodies were determined by means of a brucella agglutination test Serum dilutions from 1 10 to 1 1000 were placed in small test tubes and to each tube 1 ml of a Brucella abortus Bang antigen solution (0 2 vol) was added (made available by Dr med vet H Bendtsen Statens Veterinær Serumlaboratorium Copenhagen) The tubes were incubated at 37 C for 18 hours Agglutinin titers were expressed as the highest final serum dilution that gave a 2 plus reaction on an arbitrary scale of zero through 4-plus

RESULTS

Fig 1 shows the variations of the migration indices from 15 persons during the 18 days of the investigation Each curve has a characteristic biphasic course starting with a decrease in migra tion indices during the first 2–4 days a return to the original level about the 6th–8th day and then again a gradual decrease through the rest of the period The uniformity of this biphasic course appears clearly from Fig 1 and seems to indicate a general variation in cellular hyper sensitivity after the primary antigen stimulus

Table I shows the migration indices at dif ferent times during the immunization together with the corresponding agglutinin titers The mean

Tab e I *Corresponding values of migration indices and antibody titers after a primary immunization*

	Days after immunization													
	0		*1–2*		*4–5*		*6–8*		*9–11*		*1⌞–14*		*15–18*	
Pat no	M I	A T	M I	A T	M I	A T	M I	A T	M I	A T	M I	A T	M I	A T
1	0 93	0	0 70	0	0 87	0	0 75	0	0 71	10			0 67	40
?	1 00	0	0 63	10	0 81	0			0 68	0			0 60	5
3	0 85	0	0 64	0	1 01	0	0 91	10			0 68	90		
4	0 98	0	0 64	0	0 81	0	1 10	0			0 71	10		
5	0 90	0	0 58	10	0 83	0	0 86	100	0 67	100	0 67	⌞50		
6	0 87	0	0 74	0	0 91	0			0 73	10	0 78	⌞0		
7	0 95	0	0 56	20	0 78	0	0 91	0					0 68	50
8	0 82	0	0 70	0	0 74	0	0 90	0			0 80	20		
9	0 90	0	0 50	0	0 70	0	0 80	0	0 70	10			0 54	30
10	1 08	0	0 68	—	0 90	10			0 7	0	0 66	20	0 55	50
11	0 95	0	0 69	10	0 84	0			0 75	10	0 84	50	0 80	100
1	0 85	0	0 70	40	0 58	0	0 99	0	0 89	10	0 80	10	0 54	20
13	0 91	0	0 62	0	1 04	0			0 69	50	0 58	30		
14	0 87	0	0 74	0	0 70	0	0 98	0	0 85	60	0 70	80		
15	0 87	0	0 59	0	0 50	0	0 80	10			0 46	90	0 55	100
Mean of M I	0 9⌞±0 07		0 65±0 08		0 80±0 13		0 90±0 10		0 74±0 08		0 70±0 11		0 62±0 09	
Mean of change			−0 27±0 10		−0 11±0 16		+0 01±0 11		−0 19±0 13		−0 ⌞1±0 16		−0 33±0 11	

M I = migration index A T = antibody titer

values from each period show the aforementioned variation in the migration indices Each group is statistically different from the population at time zero ($p < 0\,001$) with the exception of the ob servations from the 6th–8th day which show the same distribution as the zero observations

In order more generally to illustrate the in dividual changes the differences are calculated between the migration index at time zero and the migration indices on the various days after im munization of each person The mean values of these differences called the means of change are also shown in Table I It appears that these mean values follow the same pattern as indicated by the variations in the mean values of the migration indices

The conclusion must be that under the chosen experimental circumstances cellular hypers nsi tivity develops during the first 1–2 days after im munization and reaches a maximum on the sec ond day The hypersensitivity then decreases and has disappeared on the 6th–8th day From the ninth day the hypersensitivity reappears and reaches approximately the same level on the 15th–18th day as was obtained on the second day

The development of humoral hypersensitivity seems to follow another course From Table I it can be seen that agglutinins do not appear until the 9th–11th day and show a gradual increase during the last part of the investigation period Only in four patients (2 7 11 and 12) were small titers of agglutinin detectable on the second day disappearing on the 4th–5th day

In accordance with earlier observations (25) it can be seen that there is no correlation between the corresponding agglutinin titers and the migra tion indices

DISCUSSION

From the present experiments at least three ob servations can be made 1 cellular hypersensitivity develops at a very early stage in the immuniza tion 2 it appears in two phases and 3 it de velops *apparently independently of humoral* hy persensitivity as expressed by agglutinating anti bodies

1 Cellular hypersensitivity is most often con sidered to develop 10–15 days after the antigenic challenge as first described by Koch (12) The o currence of shorter latent periods is however

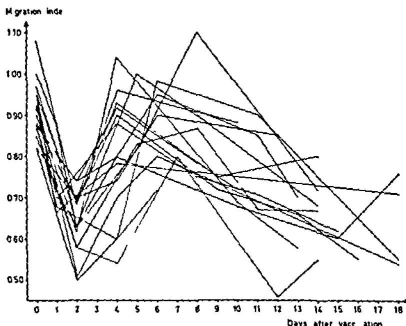


Fig 1 Variation of the migration indices in 15 persons after a primary immunization

tivity was a negative in vitro test of cellular hypersensitivity using brucella bacteria as antigen and no demonstrable titers of brucella agglutinins in the serum (see below)

As primary immune stimulus 1000 mill Brucella abortus Bang bacteria were injected subcutaneously in the thigh of each person

Determination of cellular hypersensitivity and humoral antibodies was done at the same time in each individual as described below All persons were investigated at time zero and subsequently every second and third day during the following 18 days

Demonstration of cellular hypersensitivity

The in vitro detection of cellular hypersensitivity was performed with peripheral human leucocytes according to the technique described in detail in a previous paper (24) The principle of the method is based on the inhibitory effect of an antigen upon the migration of leucocytes in vitro in case the cells originate from an organism with cellular hypersensitivity to the same antigen. The migration of the leucocytes is measured in the presence and absence of Brucella bacteria The action of antigen upon the cell migration is expressed in the so-called migration index M/M_0, where M represents the average migration area of the antigen containing cultures and M_0 the average migration area of the control cultures The migration index thus expresses the inhibition induced by the antigen in such a way that the more pronounced the inhibition the lower the migration index The Brucella bacteria were of the same kind as used in the immunization

The degree of migration inhibition has previously been shown to correlate with the degree of cellular hypersensitivity as expressed by the delayed intracutaneous reaction (5) According to these experiments a migration index of 0.78 was the borderline between brucella positive and brucella negative observations Consequently a migration index below 0.78 was the criterion of brucella hypersensitivity and persons were considered brucella negative if they had a migration index above 0.78

Demonstration of humoral hypersensitivity

Circulating antibodies were determined by means of a brucella agglutination test Serum dilutions from 1 10 to 1 1000 were placed in small test tubes and to each tube 1 ml of a Brucella abortus Bang antigen solution (0.2 vol) was added (made available by Dr med. vet. H Bendtsen Statens Veterinær Serumlaboratorium, Copenhagen) The tubes were incubated at 37 C for 18 hours Agglutinin titers were expressed as the highest final serum dilution that gave a 2 plus reaction on an arbitrary scale of zero through 4-plus

RESULTS

Fig 1 shows the variations of the migration indices from 15 persons during the 18 days of the investigation Each curve has a characteristic biphasic course starting with a decrease in migration indices during the first 2–4 days a return to the original level about the 6th–8th day and then again a gradual decrease through the rest of the period The uniformity of this biphasic course appears clearly from Fig 1 and seems to indicate a general variation in cellular hypersensitivity after the primary antigen stimulus

Table I shows the migration indices at different times during the immunization together with the corresponding agglutinin titers The mean

As soon as this migration has been accomplished the individual is in a state of "universal" hypersensitivity. The diffusion of immunologically active cells has been shown to have a pronounced peak about 4–6 days after the immunization (11) and thereafter gradually to decrease to pre-immunization levels on the 10th day. If this piece of evidence is added to the well known fact that in a steady state of hypersensitivity immunocompetent cells are found in the circulation, the conclusion might be that immunocompetent cells might be expected in high concentrations twice in the circulation, perhaps interrupted by an intermediary phase with negligible amounts of cells. As the cells in the present experiments derived only from the peripheral blood, it might be that the first phase of hypersensitivity represents cells on their way to settle down in more remote lymphatic centers where they will sensitize non-committed cells resulting in a "universal" state of hypersensitivity.

3. The last thing to be discussed is the relation between cellular and humoral hypersensitivity. During the first phase of cellular hypersensitivity detectable amounts of agglutinating antibodies are occasionally seen in very low titers. When the cellular hypersensitivity disappears, circulating antibodies are found in rising titers, later followed by increasing cellular hypersensitivity. As previously mentioned, there is no correlation between the titers and the migration indices on the different days after immunization. This finding and the discrepancy in time sequence of the appearance of agglutinating antibodies and cellular hypersensitivity might indicate that the two types of hypersensitivity are produced by different immunologic mechanisms. This simple interpretation might, however, be incorrect, as the sensitivity of the two parameters is probably very different and moreover on one side the reactivity of the cells is determined and on the other the result of cellular activity, i.e. the production of circulating antibodies. Thus experiments with the plaque forming cell technique have shown that antibody forming cells are detectable in the blood 1–2 days before the appearance of detectable amounts of circulating antibodies (8, 13, 17).

Other experiments with the plaque forming cell technique have shown that antibody forming cells develop in two peaks, the first represented by cells forming 19 S antibodies and the second by cells forming 7 S antibodies, occurring on the fourth and eleventh days respectively after the immunization (8, 29). Studies of the cell morphology connected with the immunoglobulin production have indicated that 19 S antibodies were probably associated with large basophilic cells and 7 S antibodies with plasma cells (21). No attempt was made to determine the degree of cellular hypersensitivity, but according to the immunization procedure a certain state of cellular hypersensitivity might be expected to be present.

From the fact that antibody producing cells may appear in two peaks in the same way as cells responsible for cellular hypersensitivity, it is possible that the events during the immunization may to some extent be similar, and it would be tempting to advance the hypothesis that the first phase of cellular hypersensitivity is mediated by cells able to produce both 19 S antibodies and cellular hypersensitivity. The sporadic presence of low titers of agglutinating antibodies during this phase might be an indication of a temporary production of 19 S antibodies which are known to precede the production of 7 S antibodies in brucella hypersensitivity (30). Unfortunately the titers have been too low to determine the class of immunoglobulins in the present experiments.

In conclusion it may be said that the experiments have shown that cellular hypersensitivity to brucella bacteria develops in two phases. This is possibly an expression of a general immunologic phenomenon following any primary immunization. It is not possible from the presented data to give an explanation of the immunological mechanisms behind this biphasic curve or to elucidate much further the interrelationship between humoral and cellular hypersensitivity. The described experimental model together with other more advanced immunological techniques seem, however, to offer good possibilities of studying these very interesting problems more closely.

REFERENCES

1. Barnes, D. W. H. & Loutit, J. F.: Lancet 2: 1138, 1967.
2. Bouquet, A.: Ann Inst Pasteur 66: 1, 1941.
3. Brent, L. & Medawar, P. B.: Proc roy Soc B 165: 81, 1966.
4. Burnet, F. M.: Aust Ann Med 11: 79, 1962.
5. Coe, J. L. & Salvin, S. B.: J Immunol 93: 495, 1964.

6 Debré R. Paruf J & Dautrebande L Ann. Méd 9 443 1921
7 Dienes L & Mallory T B Proc Soc exp Biol (N Y) 34 59 1936
8 Eidinger D & Pross H F J exp Med 126 15 1967
9 Gell P G H & Hinde J T Int Arch Allergy 5 23 1957
10 Gowans J L & McGregor D D Progr Allergy 9 1 1965
11 Hall J G Morris B Moreno G D & Bessis M C J exp Med 125 91 1967
12 Koch R Dtsch med Wschr 17 101 1891
13 Landy M Sanderson, R. P & Jackson A. L. J exp Med 122 483 1965
14 Metaxas M N & Metaxas-Buhler M Schweiz. Z allg. Path 17 128 1954
15 Micklem H S Ford C E Evans E P & Gray J Proc roy Soc B 165 78 1966
16 Mote J R & Jones T D J Immunol 30 149 1936
17 Moller G & Wigzell H J exp Med 121 969 1965
18 Oort J & Turk J L Brit J exp Biol 46 147 1965
19 Prendergast R A J exp Med 119 377 1964
20 Salvin S B J exp Med 107 109 1958
21 Schoenberg M D Stavitsky A B Moore R. D & Freeman M J J exp Med 121 577 1965
22 Sell S & Weigle W O J Immunol 93 495 1959
23 Simon F A & Rackemann F M J Allergy 5 439 1934
24 Søborg M & Bendixen G Acta med scand 181 247 1967
25 Søborg M Acta med scand 18. 167 1967
26 Tremaine M M & Jeter W S J Immunol 74 96 1955
27 Turk J L & Stone S H In Cell bound antibodies (eds B Amos and H Koprowski) p 51 Wistar Institute Press Philadelphia 1963
28 Uhr J W Salvin S B & Pappenheimer A M J exp Med 105 11 1957
29 Wigzell H J J exp Med 124 953 1966
30 Wilkinson P C J Immunol 96 457 1966

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EFFECT OF A NEW ADRENERGIC β BLOCKING AGENT H 56/28 ON NERVOUS HEART COMPLAINTS

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Abstract The effect of a new adrenergic β receptor blocking agent H 56/28 has been studied in fourteen patients with nervous heart complaints mainly palpitations Organic heart disease and hyperthyroidism were excluded The investigation was performed as a double blind test During a first treatment period half of the patients received H 56/28 and the other half placebo-tablets during the second period the treatment was reversed The patients were also studied during repeated exercise tests The heart rate during exercise was significantly reduced by H 56/28 and there was also a sl ght though not significant decrease of the systolic blood pressure In addition to the immediate effect H 56/28 treatment seemed to have a more prolonged lowering effect on the heart rate No side effects of the drug worth mentioning were seen All but three patients preferred H 56/28 to placebo The β blocker was especially effective in relieving palpitations It is concluded that β blocking agents might be of value in treating patients with nervous heart complaints

Several authors (1 3 4 10 11) have found β adrenergic blockade effective against nervous heart complaints Not only the palpitations and other chest discomfort but also the anxiety were relieved All these investigations were performed with propranolol Another β receptor blocking agent with slightly different pharmacological properties H 56/28 (1-(o allylphenoxy) 3 isopropylamino 2 propanol hydrochloride) has recently been described (8 9 12) In addition to its β blocking effect it is reported to have a weak β stimulating effect In the present investigation trials were made with this new drug on an outpatient group with subjective cardiac symptoms where no signs of disease of the heart could be detected The patients were studied both in rest and exercise

MATERIAL AND METHODS

Fourteen outpatients have been investigated five men aged 21 to 38 (mean 9) and nine women aged 17 to 5? (mean 31) The chief complaint of all the patients was palpitations including rapid and heavy action of the heart As seen in Table I many of them also had a feeling of oppression or pain in the chest as well as nervousness sweating fatigue and dizziness Pain in the chest and oppression were in all cases mild and not typical of coronary heart disease None of the patients had hyperthyroidism

The investigation was performed on a double bl nd basis (Fig 1) At the first visit two exercise tolerance tests were carried out one before (E_1) and another (E) one hour after oral intake of 80 mg of H 56/28 The patients rested between the tests During the succeeding two to three weeks the patients received tablets marked A After this period they returned for a second visit without having taken tablets for the last 24 hours Again two exercise tests were performed before (E) and one hour after (E) taking their own tablets marked A After a s cond period of two to three weeks when the patients were given tablets marked B they performed exercise tests in the same manner before (E) and one hour after (E) oral intake of their tablets marked B

The tablets marked A given to group 1 (seven patients) contained H 56/?8 (20 mg) of which they received 80 mg four times daily during the first treatment period and 80 mg before the second exercise test (E) at their second visit to the laboratory During the second treatment period they received placebo Group 2 (seven patients) received placebo during their first treatment period H 56/28 during the second period Instead of the above mentioned dose the first four patients took 40 mg of H 56/28 four times daily and 40 mg of H 56/28 before the exercise tests

The exercise tolerance tests were carried out with the subjects in the sitting posit on on an electrically braked bicycle ergometer (7) The first load was 200 kpm/min for women and 300 kpm/m n for men The work load was then increased by 200 kpm/min for women and by 300 kpm/min for men every 4th or 6th minute when the patient had reached a relative circulatory steady state i e an increase in the heart rate of no more than four beats in two minutes

During the exercise tests heart rate was estimated in the ECG every second minute Blood pressure was measured indirectly in the arm Before the investigation and at each succeeding visit the patients underwent routine

Table I *Symptoms before and during the two treatment periods*

		Symptoms					
		H 56/28 period			Placebo period		
	First visit	+	0	–	+	0	–
Palpitations	14	14	0	0	5	8	1
Pain in chest	9	5	4	0	3	5	1
Oppression	9	8	1	0	2	7	0
Breathlessness	6	5	1	0	3	3	0
Sweating	10	6	3	1	4	5	1
Tremor	6	6	0	0	2	4	0
Nervousness	11	6	5	0	3	7	1
Headache	7	6	1	0	3	3	1
Depression	7	7	0	0	5	2	0
Fatigue	10	6	4	0	4	6	0
Dizziness	9	6	3	0	4	4	1
Gastro-intestinal disturbances	4	1	1	2	2	1	1

+ = improved 0 = unchanged – = impaired

physical examination blood samples were drawn for analysis of ESR, Hb WBC differential leucocyte count, thrombocytes bilirubin, thymol reaction alkaline phos phatases, glutamate oxalacetate transaminase (GOT) glutamate pyruvate transaminase (GPT) lactate dehydrogenase isoenzyme determination (LDH-electrophoresis) creatinine and cholesterol urine samples were taken for analysis of protein and glucose and for microscopical ex amination. Before the investigation basal metabolic rate and proteinbound iodine were checked. X ray controls of the heart and lungs were also made

RESULTS

1 *Subjective Symptoms*

Almost throughout H 56/28 had a positive effect as compared with that of the placebo both regarding somatic and psychic symptoms H 56/28 was especially effective in relieving palpitations an effect which was seen in all the patients Table I shows the effect of H 56/28 on the other symptoms

In some cases the placebo had a positive effect This was independent of whether or not the patients had received H 56/28 during a preceding period

Two patients have reported gastrointestinal side effects mainly nausea One of them had however nausea also during the placebo period After lowering the placebo dose from 4 to 2 tablets four times daily and the H 56/28 dose from 4 to 3 tablets four times daily these side effects disappeared Another patient had nausea after H 56/28 2 tablets four times daily when taking them before meals but did not feel sick when taking them after she had eaten One patient complained of slight night sweating during treatment with H 56/28 which she had not experienced before the trial

On asking the patients at the end of the trial which tablet (A or B) they preferred all except three preferred H 56/28 One felt sick when taking H 56/28 before meals (see above) and therefore she preferred the placebo She was given a low dosage of 2 tablets four times daily and reported that both H 56/28 and placebo had a good effect on her original subjective symptoms Another patient after great hesitation stated that she found the placebo preferable as it had been more effective than H 56/28 on the heavy heart action However H 56/28 had

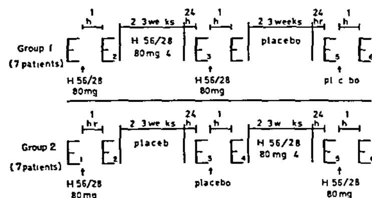


Fig 1 The investigation was performed as a double blind test Group 1 received H 56 8 during the first and placebo during the second treatment period In group the treatment periods were reversed Before and after these treatment periods two exercise tests (*E*) were made Between these exercise tests H 56/28 or placebo was given

had a good effect on her tremor vertigo and rapid heart action A third patient who had previously been a moderate smoker could not decide which tablet was better He felt better during the second treatment period when he got H 56/28 but thought that this was because he had begun to smoke again at the beginning of this period

2 *Physical Examination*

Nine patients had a systolic apical murmur or a systolic murmur in the left second intercostal space always very discrete short and protosystolic These murmurs have not been considered pathological No changes in the physical condition of the heart were noted during the trial except for changes in heart rate and blood pressure which are described below

On the first examination six patients had slightly moist and warm skin and four had finger tremor After the placebo period these findings disappeared in half of these patients After treatment with H 56/28 all the patients were normal in these respects Apart from this the physical examination did not reveal any notable findings either before or during the trial

3 *Laboratory Tests*

Before the trial was started one patient had GOT 49 and GPT 57 units and also a pathological LDH electrophoresis causing suspicion of liver damage At controls after three and six weeks (placebo during the first period) GOT values were normal but GPT values were 76 and 75 units respectively and LDH-electrophoresis was unchanged The other liver function tests and ESR were normal Fine needle liver biopsy showed a normal cytological picture The findings have been interpreted as remains of a subclinical hepatitis some time before the trial It should be pointed out, that this patient had had his heart symptoms for several years which makes it rather improbable that the positive effect experienced during the H 56/28 period could be due to spontaneous regression This patient also had a long history of orthostatic albuminuria which remained unchanged during the trial

Another patient had an elevated GPT value 61 units after the H 56/28 period whereas it had been normal before the trial and after the placebo given during the first treatment period

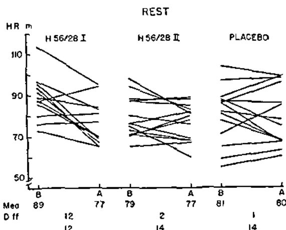


Fig 2 Heart rate of each patient at rest before (*B*) and one hour after (*A*) H 56/28 or placebo The examinations represented by the middle (H 56/28 II) and the right band (placebo) part of the figure were preceded by treatment periods with the drug or placebo Two patients were excluded at the first examination (H 56 28 I) for technical reasons

GOT LDH-electrophoresis ESR and the other laboratory tests were however normal during the whole time Three months later the patient still had GPT 58 units The other liver function tests were normal During these months he had been taking propranolol (20 mg four times daily) A few weeks later also GPT was normalised

There were no other pathological findings during the trial

X ray examination of the heart and lungs was normal in all patients

4 *Heart Rate and Arterial Blood Pressure*

Rest

The heart rate at rest before the first exercise test (E_1 Fig 1) varied between 73 and 114 beats/min mean 89/min (Fig 2) Thus not all patients had tachycardia though all complained of palpitations At the two exercise tests (E_3 and E_5) after the treatment periods the mean resting heart rate was about 10 beats/min lower than at the first time (E_1) and there was no difference between the heart rate at rest after the treatment period with H 56/28 and after that with the placebo

One hour after H 56/28 was given for the first time (before the second exercise test E) the mean resting heart rate was 12 beats lower ($p < 0.02$) than before but the next time H 56/28 was taken there seemed to be no effect on the heart rate at

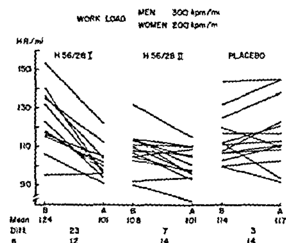


Fig 3 Heart rate during low work load before (*B*) and one hour after (*A*) H 56/28 or placebo The examinations represented by the middle (H 56/28 II) and the right hand (placebo) part of the figure were preceded by treatment periods with the drug or placebo Two patients were excluded at the first examination (H 56/28 I) for technical reasons

rest Nor was this the case after placebo (Figs 2 and 5) Thus it can be concluded that an exercise test does not seem to influence the resting heart rate one hour later H 56/28 seems also to have little effect on the heart rate at rest when the patients are under more basal conditions (cf 8) which they probably were not the first time the drug was tested

One patient had slightly elevated blood pressure (160–175/95) at rest while the others had normal pressures At the two examinations after the treatment periods the mean resting systolic blood pressure was 7 mm Hg lower (Fig 7) and the diastolic pressure was virtually unchanged The immediate effect of H 56/28 was on the two occasions when it was examined a slight though not significant decrease of the resting systolic blood pressure (Fig 7) while the diastolic blood pressure remained unchanged The placebo changed neither the systolic nor the diastolic blood pressure which implies that the exercise test performed between the two measurements did not influence the blood pressure

Exercise

Although many of the patients suffered from tiredness only a few of them had a somewhat low working capacity One male (28 y) did not manage 900 kpm/min and three females (23 43 and 52 y) were not able to work on 600 kpm/min at a relatively steady state

The main immediate effect of H 56/28 on the working capacity was that on several occasions the patients tried a higher working load than before In four cases they were able to do this at steady state (cf 2 3 and 5) After the treatment periods (E_2 E_3) six patients were able to work at a higher load at steady state In five of the patients however this could not be attributed to H 56/28 as it was achieved also after the placebo period which in these cases was the first treatment period Two of these six patients reported that they had increased their physical activity during the treatment In a few other cases after treatment with H 56/28 the patients tried a higher work load than at the first time but without reaching a steady state

With only a few exceptions the heart rate during exercise was lowered after H 56/28 as has been reported for other β blocking drugs The effect was more marked at higher work loads i e at higher heart rates (Figs 3 4 and 5) The second time H 56/28 was tried during an exercise test i e after the H 56/28 treatment period the decrease of the exercise pulse was less noticeable This might be due to the fact that the heart rate during the exercise test before the drug was administered was lower than at the first test (E_1) At the two exercise tests one hour after H 56/28

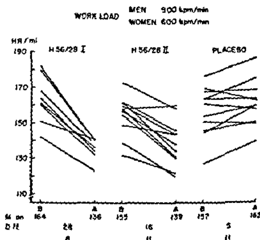


Fig 4 Heart rate during high work load before (*B*) and one hour after (*A*) H 56/28 or placebo The examinations represented by the middle (H 56/28 II) and the right hand (placebo) part of the figure were preceded by treatment periods with the drug or placebo

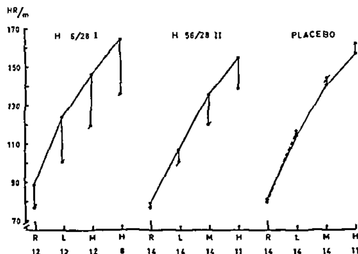


Fig 5 Mean heart rate at rest and during increasing work load before (——) and one hour after (---) H 56/28 and placebo The examinations represented by the middle (H 56/28 II) and the right hand (placebo) part of the figure were preceded by treat ment periods with the drug or placebo *R* rest *L* low (?00 or 300 kpm/min) *M* medium (400 or 600 kpm/min) *H* high (600 or 900 kpm/min) work load

the mean heart rates for each work load were almost identical The mean heart rates at the two exercise tests after the placebo period were very similar being only slightly higher the second time (+5 beats/min for the highest work load) Thus the first exercise influenced the second very little

Fig 6 summarizes the "chronic effect of H 56/28 on the heart rate at rest and during exercise The patients are divided into two groups 1 and 2 according to the order of the treatment periods The mean values are based on the first exercise test (E_1 E_3 and E_5) at each of the three examina

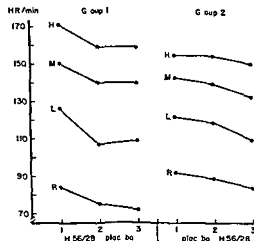


Fig 6 Mean heart rate at rest (*R*) and low (*L*) medium (*M*) and high (*H*) work load at the first exercise test (E E and E_5) at each of the three examinations The patients were divided into groups 1 and 2 according to the order of the treatment periods

tions As can be seen the entire decrease of the heart rate in group 1 was achieved during the H 56/28 period (except at rest) while in group 2 the lowering of the heart rate occurred in two steps" the second (corresponding to the H 56/28 period) being slightly bigger than the first The difference between the two curves will be discussed later

H 56/28 lowered not only the heart rate dur ing exercise but also the systolic blood pressure (the diastolic pressure will not be discussed as its auscultatory measurement is rather unreliable during exercise) The first time the drug was taken the effect seemed to be more marked the higher the pressure but this was not noticeable the second time (Fig 7) After placebo the systolic blood pressure during exercise was virtually unchanged

5 ECG

At rest all ECGs were normal except in four pa tients where slightly depressed T waves were seen In two of these the ECGs were normalized one hour after H 56/28 In one of these patients the ECG was normal also one hour after the placebo In four cases with a normal ECG at rest the amplitude of the T waves increased one hour after H 56/28 In one patient this was seen also after the placebo

During and shortly after exercise three patients showed slight ST T-changes which were not re garded as a sign of coronary insufficiency These changes were not noticeably influenced by H 56/28 or placebo

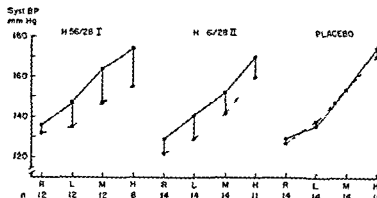


Fig 7 Mean values for systolic blood pressure at rest and during increasing work load before (——) and one hour after (---) H 56/28 and placebo The examinations re presented by the middle (H 56/28 II) and the right hand (placebo) part of the figure were preceded by treatment periods with the drug or placebo *R* rest *L* low (200 or 300 kpm/min) *M* medium (400 or 600 kpm/min) *H* high (600 or 900 kpm/min) work load

DISCUSSION

The patients studied in the present investigation must be considered a rather heterogeneous group However they all had one complaint in common palpitations of the heart—the symptom which brought them to the doctor Many of the patients also experienced pain in the chest and oppression which were invariably of vague and unspecific character These symptoms like all the others gave the impression of being nervous or functional in origin Coronary and other organic heart diseases as well as hyperthyroidism could in all probability be excluded by means of routine examinations laboratory tests X ray and ECG at rest and during work As only few of the patients had a low physical working capacity the majority could not be said to represent the condition known as vasoregulatory asthenia (VA) (6) even if some of them probably belonged to this group Furberg (5) has recently shown however that some changes of vasoregulation typical of VA patients may be found in subjects with a high physical working capacity Bollinger et al (3) has also described a group of patients with neurovegetative resting tachycardia without impairment of working capacity

Though the group of patients studied was not homogeneous it was nonetheless fairly representative of the category of patients who seek medical advice for nervous heart complaints especially palpitations It should be noted that not all patients who complain of palpitations have continuous or sporadic true tachycardia but rather define their suffering as a forceful or heavy action of the heart Most of our patients had both a heavy and a rapid heart action It seems quite reasonable that those who had tachycardia felt better during the treatment with H 56/28 as one of the main effects of β blocking agents is reduction of the heart rate It has recently been shown that H 56/28 is effective against sinus tachycardia (9) It was also well established in the present study that H 56/28 reduces the heart rate especially during exercise The condition of those hwo complained of a predominantly heavy action of the heart was also improved however by H 56/28 which is interesting but less easily explained It might be due to a negative inotropic effect of the drug This property of H 56/28 is said to be weak in normal individuals (12) but might be of importance in this group of patients

Concerning the subjective symptoms all but three patients preferred H 56/28 to the placebo The objective chronic effect of H 56/28 and the placebo also needs some consideration The decrease in heart rate which was observed especially after the H 56/28 periods might be caused not only by this treatment but by other factors as well The lowering of the heart rate in group 2 at rest and during exercise after the first period (placebo period) was most probably due to psychic factors e g better knowledge of the experimental conditions or a feeling of being taken care of This must imply that the decrease in heart rate in group 1 after the first period (H 56/28 period) was caused not only by H 56/28 but also by the psychic factors mentioned above Similar psychic conditions probably existed at the third visit This is also suggested by the curves for group 1 where there was no further decrease in heart rate (except at rest) after the placebo period

Physical training during the treatment periods could also cause a decrease in heart rate but this

factor is probably of less importance as all except two patients denied any increase in their physical activity during the treatment period In these two cases it cannot of course be excluded that physical training may partly explain the decrease in heart rate during exercise

Another explanation of the lower heart rate after the H 56/28 period might be that the last dosage of H 56/28 taken the previous day could still be effective This seems rather improbable however as the heart rate was very similar also after the placebo period in group 1

Thus it can be said that after treatment with H 56/28 for a couple of weeks the effect on the heart rate at rest and during exercise persisted for some time and that probably this effect can not be completely explained by psychic factors and the result of physical training How H 56/28 exerts its influence in this respect can only be however a matter of speculation but the pos sibility that during the treatment period a vicious circle has been broken should nevertheless be mentioned

On the basis of the present investigation it seems reasonable to assume that β blo king agents can be valuable in the treatment of patients with nervous heart complaints

REFERENCES

1 Besterman E M M & Friedlander D H Postgrad med J 41 5_6 1965
2 Bollinger A Gander M & Forster G Schweiz med Wschr 95 1075 1965
3 Bollinger A (ander M Pylkkanen, P O & Forster G Cardiolo_ia (Basel) Suppl _ 68 1966
4 Frohlich E D Dustan H P & Page I H Arch intern Med 177 614 1966
5 Furberg G Acta med scand 18 119 1967
6 Holmgren A Jonsson B Levander M Linderholm, H, Sjostrand, T & Strom G. Acta med scand 158 413 1957
7 Holmgren A & Mattsson K H Scand J clin Lab Invest 6 137 1954
8 Johnsson G Norrby L Solvell L & Ablad B Lak Tidn Suppl 1 47 1966
9 Linko E, Sutonen, L & Ruosteenoja, R Acta med scand 181 547 1967
10 Nordenfelt O Acta med scand 178 393 1965
11 Turner P, Granville-Grossman K L & Smart J V Lancet 2 1316 1965
1_ Ablad B Brogård, M & Ek L Acta pharmacol (Kbh) Suppl 2 9 1967

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CLINICAL USE OF HIGH DOSES OF FUROSEMIDE (LASIX®) IN THE TREATMENT OF RESISTANT NEPHROTIC EDEMA

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Abstract Four cases of the nephrotic syndrome are reported in which furosemide was administered in doses higher than those usually prescribed In three cases merely raising the dose of furosemide to levels up to 600 mg/day promoted a diuresis In one patient furosemide in doses up to 1200 mg/day failed to produce a diuresis but the addition of a thiazide succeeded in producing one Hypokalemia was seen in three patients and was treated by reduction of the drug dose oral administration of potassium chloride and spironolactone In cases of edema resistant to the usual doses of furosemide the use of higher doses alone or in combination with other diuretic agents may produce a satisfactory diuretic response

Furosemide N-(2 furylmethyl)-4-chlor 5-sulfamoyl anthranilic acid is a relatively new diuretic that has been shown to be very effective in the treatment of edema resistant to other diuretic therapy (5 7 9 14 15) Patients are occasionally seen however who do not respond satisfactorily to combinations of furosemide and other diuretic agents in their usually recommended doses In such patients the administration of much higher doses of furosemide (3 5 7 14) even up to 1800 mg/day (5 14) have been required to promote a diuresis

This report concerns four patients with resistant edema secondary to the nephrotic syndrome in whom high doses of furosemide alone and in combination with other diuretics were administered when the usual doses failed to promote a diuresis

MATERIAL AND METHODS

All four patients were adults and all showed membranous glomerulonephritis on renal biopsy One patient had diabetes and one had lupus erythematosus. The other two had the nephrotic syndrome without associated diseases and with no history of a preceding acute glomerulonephritis Furosemide was administered in tablet form, each tablet containing 40 mg. The frequency of administration varied from 1 to 5 times daily All patients were hospitalized throughout the study and all were restricted in sodium intake to 22 mEq/day Observations made included body weight, urine volume serum potassium, chloride and creatinine blood urea nitrogen, blood uric acid blood pH serum bilirubin, serum glutamicoxaloacetic acid transaminase (SGOT) serum glutamic pyruvic acid transaminase (SGPT) serum alkaline phosphatase hemoglobin, red cell count, differential and platelet count. Twenty four hour collections of urine were analysed for sodium, chloride potassium and creatinine

CASE REPORTS

Case 1

Diabetes and membranous glomerulonephritis (Fig 1) A 28 year-old woman was first seen at the Renal Clinic in March 1965 She had had diabetes since June 1964 and had been maintained on about 28 units of lente insulin daily since that time On this dose the fasting blood sugar remained around 125 mg/100 ml. Proteinuria was first noted in November 1964 and in January 1965 she began to note swelling of her legs When seen at the Renal Clinic in March 1965 edema was noted in both legs up to the groin, and small pleural effusions were present bilaterally The B.P was 160/90 and the eye grounds were normal. The rest of the physical examination was unremarkable The laboratory values are recorded in Table I Two LE clot tests and an excretory urogram were normal. A renal biopsy revealed membranous glomerulonephritis

Over the next 15 months the patient received a combination of polythiazide 4 mg/day spironolactone 25 mg three times daily prednisone 5 mg three times daily mannitol and albumin infusions, and two seven-day courses of ACTH injections On this treatment the edema decreased but never disappeared entirely In June 1966 a marked increase in weight was noted, and did not respond to a combination of methylprednisolone 20 mg twice daily Prednisolone was discontinued and azathioprine 100 mg daily was administered. Because of severe fluid retention scarification was performed but the patient continued to gain weight. Peritoneal dialysis was started on November 28 1966 the patient's weight falling from 67.0 to 55.4 kg during this three-day procedure After

Table I *Summary of blood chemistry of the four cases*

Case	1			2			3		4	Normal values
Date	3 65	11/66	1/67	10 65	10 66	1/67	6 66	1/67	11/66	
BUN	61	35	35	36	44	29	46	105	113	5 [illegible]0 mg/100 ml
Serum creatinine	1 7	1 3	2 7	1 4	2 5	[illegible] 5	1 65	5 2	3 45	0 6–1 2 mg/100 ml
Creatinine clearance	39	14	13	5[illegible]	44	45	54	14	[illegible]0	70–150 ml min
Hemoglobin	13 8	8 7	9 2	14 0	15 1	14 0	11 0	9 3	13 3	11 5–17 2 g/100 ml
Blood cholesterol	905	880	880	60[illegible]	618	600	518	538	860	150–275 mg/100 ml
Serum albumin	1 5	0 7	1 0	1 8	1 8	1 7	2 2	2 3	2 7	4 8–5 8 g/100 ml
α 1 globulin	0 23	0 23	0 71	0 [illegible]9	0 29	0 28	0 35	0 35	0 2[illegible]	0 20–0 32 g/100 ml
α 2 globulin	1 38	1 86	1 50	0 75	0 63	0 67	0 4	0 47	0 84	0 32–0 54 g/100 ml
β-globulin	0 92	0 98	1 33	0 73	0 73	0 74	0 82	0 86	0 71	0 43–0 85 g/100 ml
γ-globulin	0 56	0 5[illegible]	0 50	0 34	0 28	0 29	0 72	0 75	0 46	0 61–1 17 g/100 ml
Blood sugar	125	1[illegible]0	122	99	90	98	86	84	100	70–110 mg/100 ml
Serum Na	133	13[illegible]	138	13[illegible]	130	138	132	134	134	133–141 mEq/l
Serum K	3 8	3 8	3 4	3 8	3 7	2 7	4 4	4 4	4 4	3 3–4 4 mEq/l
Serum Cl	98	10[illegible]	106	102	101	105	104	106	102	97–108 mEq/l

dialysis however the patient s weight gradually increased again despite furosemide 80 mg twice daily and spironolactone 25 mg three times daily She was again hospitalized on January 11 1967 and her hospital course can be seen in Fig 1 and Table 1

There was no diuretic response or loss of weight until the dose of furosemide reached 1 0 mg three times daily At this point a slight diuresis was noted When the dose was raised to 160 mg three times daily a marked diuresis occurred The serum potassium was 3 4 mEq/l and fell to [illegible]9 mEq l after one day on the high dose of furosemid despite the use of potassium supplements and spironolactone Gradually over the next two weeks the serum potassium returned to normal Little change was noted in the blood pH BUN serum creatinine or creatinine clearance The blood sugar and insulin requirements during treatment did not change The serum uric acid level was normal throughout the entire course No changes in alkaline phosphatase serum bilirubin SGOT SGPT hemoglobin white cell or platelet counts were noted Gradually over the next three months the patient was able to discontinue spironolactone and to reduce furosemide to 40 mg twice daily

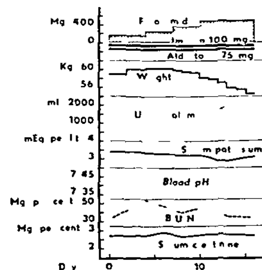


Fig 1 Case 1 [illegible]8 year-old woman. Diabetes and membranous glomerulonephritis

Case

Membranous glomerulonephritis (Fig [illegible])

A 56-year-old man was first seen at the Renal Clinic in October 1965 In January 1964 he had noted a gradual onset of swelling in his legs and face Slight proteinuria and mild hypertension were found at that time He was treated with thiazide diuretics and the swelling disappeared in one week In September 1965 he again began to note the gradual onset of swelling in his face and legs When seen at the Renal Clinic in October 1965 moderate leg edema was found and pleural effusions were noted bilaterally The B P was [illegible]30/140 and marked arteriolar spasm without hemorrhages or exudates was noted in the eye grounds The rest of the physical examination was unremarkable The laboratory values are recorded in Table 1 Two LE clot tests and an excretory urogram were normal The patient was treated with prednisone 10 mg three times daily and chlorthalidon 100 mg dail On this treatment his edema disappeared within four months and the medications were discontinued

In September 1966 he again noted gradual onset of swelling in his legs and face In October 1966 physical examination revealed marked leg edema and a B P of 170 1 0 A renal biopsy revealed membranous glomerulonephritis The laboratory values are recorded in Table I Over the next two months despite treatment with furosemide 160 mg twice daily and spironolactone 5 mg four times daily his weight gradually rose from 67.0 to 75 9 kg

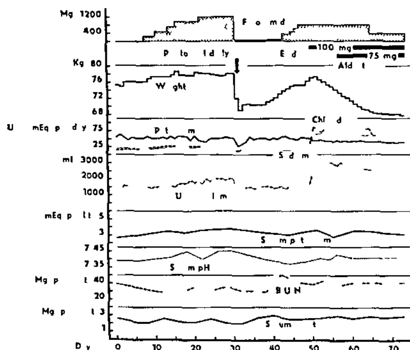


Fig 2 Case 2 56-year-old man Membranous glomerulonephritis

In January 1967 the patient was hospitalized again and his course can be seen in Fig 2 and Table I Over a period of two weeks the dose of furosemide was gradually increased to 400 mg three times daily but even after nine days of treatment with this dose no diuretic response occurred and severe fluid retention prompted peritoneal dialysis The patient's weight fell from 78 1 to 69 0 kg during this three day procedure Gradually over the next four weeks however despite treatment with furosemide in doses up to 160 mg five times daily no diuretic response occurred When hydrochlorothiazide 50 mg twice daily was added an immediate and brisk diuresis occurred Within a few days a moderate hypokaliemia developed with serum potassium falling to 2 8 mEq/l The addition of spironolactone 75 mg three times daily raised the serum potassium to 3 5 mEq/l over the next three days and the potassium values thereafter stayed between 3 1 and 3 5 mEq/l When the dose of furosemide was reduced to 80 mg five times daily an immediate decrease in sodium and chloride excretion was noted although there was little change in potassium excretion Throughout the whole period little change was noted in the blood pH BUN serum creatinine and creatinine clearance Both the fasting blood sugar and uric acid levels remained normal and unchanged throughout the study No changes in the serum alkaline phosphatase serum bilirubin SGOT SGPT hemoglobin white cell or platelet counts were noted Gradually over the next three months the patient was able to discontinue spironolactone and the thiazides and to reduce the dose of furosemide to 40 mg twice daily

Case 3

Lupus erythematosus and membranous glomerulonephritis (Fig 3 Table I)

A 6? year-old man was first seen at the Renal Clinic in June 1966 He had had discoid lupus erythematosus since 1933 and intermittent proteinuria since 1945 In April 1966 he noted the gradual onset of swelling in both legs He was given chlorthalidon 100 mg daily but this was discontinued after seven weeks because it had no effect and made his skin lesions worse From April until June 1966 the swelling gradually increased When seen at the Renal Clinic at this time he was noted to have typical discoid lupus erythematosus lesions on his face and abdomen Moderate edema was noted in both legs up to the groin The BP was 175/95 and the eye grounds were normal The rest of the physical examination was unremarkable The laboratory values are recorded in Table I Two LE clot tests were positive An excretory urogram was normal A renal biopsy revealed membranous glomerulonephritis

The patient was given a ?2 mEq Na diet but, because of his known sensitivity to medication no additional treatment was given Over the next four months his edema steadily increased He was seen again in November 1966 at which time it was noted that his creatinine clearance had decreased to 14 ml/min He was given 80 mg of furosemide once daily and prednisone 10 mg twice daily Despite this regimen the patient continued to gain weight and in January 1967 he was hospitalized His course in the hospital can be seen in Fig 3 and Table I

When the dose of furosemide was increased to 200 mg three times daily a moderate diuretic response occurred Increasing the furosemide to 00 mg four times daily failed to increase the response further When spironolactone was added in a dose of 5 mg four times daily an immediate increase in diuresis occurred The addition of chlorothiazide at a later time increased the diuresis

Table I *Summary of blood chemistry of the four cases*

Case	1			2			3		4	Normal values
Date	3 65	11/66	1 67	10 65	10 66	1 67	6 66	1/67	11/66	
BUN	61	15	35	36	44	29	46	105	153	5–20 mg 100 ml
Serum creatinine	1 7	1 3	2 7	1 4	2 5	2 5	1 65	5 2	3 45	0 6–1 2 mg 100 ml
Creatinine clearance	39	14	13	52	44	45	54	14	20	70–150 ml min
Hemoglobin	13 8	8 7	9 2	14 0	15 1	14 0	11 0	9 3	13 3	11 5 17 2 g 100 ml
Blood cholesterol	905	880	880	602	618	600	518	538	860	150–275 mg 100 ml
Serum albumin	1 5	0 7	1 0	1 8	1 8	1 7	2 2	2 3	2 7	4 8–5 8 g 100 ml
α 1 globulin	0 [illegible]3	0 23	0 [illegible]	0 29	0 [illegible]9	0 28	0 35	0 35	0 2[illegible]	0 20–0 3[illegible] g 100 ml
α globulin	1 38	1 86	1 50	0 75	0 63	0 67	0 4[illegible]	0 47	0 84	0 37–0 54 g/100 ml
β-globulin	0 9[illegible]	0 98	1 33	0 73	0 73	0 74	0 8	0 86	0 71	0 43–0 85 g 100 ml
γ globulin	0 56	0 52	0 50	0 34	0 [illegible]8	0 29	0 7[illegible]	0 75	0 46	0 61–1 17 g 100 ml
Blood sugar	125	1[illegible]0	12[illegible]	99	90	98	86	84	100	70–110 mg 100 ml
Serum Na	133	13[illegible]	138	13[illegible]	130	138	132	134	134	133–141 mEq/l
Serum K	3 8	3 8	3 4	3 8	3 7	2 7	4 4	4 4	4 4	3 3–4 4 mEq l
Serum Cl	98	102	106	102	101	105	104	106	102	97–108 mEq/l

dialysis however the patient s weight gradually increased again despite furosemide 80 mg twice daily and spironolactone [illegible]5 mg three times daily She was again hospitalized on January 11 1967 and her hospital course can be seen in Fig. 1 and Table I

There was no diuretic response or loss of weight until the dose of furosemide reached 120 mg three times daily At this point a slight diuresis was noted When the dose was raised to 160 mg three times daily a marked diuresis occurred The serum potassium was 3 4 mEq l and fell to [illegible] 9 mEq l after one day on the high dose of furosemide despite the use of potassium supplements and spironolactone Gradually over the next two weeks the serum potassium returned to normal Little change was noted in the blood pH BUN serum creatinine or creatinine clearance The blood sugar and insulin requirements during treatment did not change The serum uric acid level was normal throughout the entire course No changes in alkaline phosphatase serum bilirubin SGOT SGPT hemoglobin white cell or platelet counts were noted. Gradually over the next three months the patient was able to discontinue spironolactone and to reduce furosemide to 40 mg twice daily

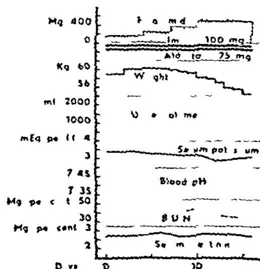


Fig 1 Case 1 [illegible]8 year-old woman Diabetes and membranous glomerulonephritis.

Case 2

Membranous glomerulonephritis (Fig 2)

A 56-year-old man was first seen at the Renal Clinic in October 1965 In January 1964 he had noted a gradual onset of swelling in his legs and face Slight proteinuria and mild hypertension were found at that time He was treated with thiazide diuretics and the swelling disappeared in one week In September 1965 he again began to note the gradual onset of swelling in his face and legs When seen at the Renal Clinic in October 196[illegible] moderate leg edema was found and pleural effusions were noted bilaterally The B P was 230/140 and marked arteriolar spasm without hemorrhages or exudates was noted in the eye grounds The rest of the physical examination was unremarkable The laboratory values are recorded in Table I Two LE clot tests and an excretory urogram were normal The patient was treated with prednisone 10 mg three times daily and chlorthalidon 100 mg daily On this treatment his edema disappeared within four months and the medications were discontinued

In September 1966 he again noted gradual onset of swelling in his legs and face In October 1966 physical examination revealed marked leg edema and a B P of 170 1 0 A renal biopsy revealed membranous glomerulonephritis The laboratory values are recorded in Table I Over the next two months despite treatment with furosemide 160 mg twice daily and spironolactone 5 mg four times daily his weight gradually rose from 67.0 to 7[illegible]9 kg.

rising to 7 68 Spironolactone in a dose of 25 mg three times daily was administered and furosemide was decreased and finally discontinued for four days With improvement in the hypokalemic alkalosis the drug was again started in a dose of 80 mg twice daily On this dose the patient s edema gradually increased over the next two months and only disappeared when the dose was raised to 120 mg twice daily A marked improvement in the BUN serum creatinine and creatinine clearance was noted during the course of therapy The blood sugar and uric acid levels were normal and unchanged during the course of therapy No changes in the alkaline phosphatase serum bilirubin SGOT SGPT hemoglobin white cell or platelet counts were noted

DISCUSSION

When no diuretic response occurs with the usual doses of a diuretic the tendency is to add another diuretic agent to the therapeutic program rather than to increase the dose of the original agent Recent experience with furosemide and ethacrynic acid (5 7 14) indicates that merely raising the doses of these drugs may at times produce a diuresis when usual doses are unsuccessful The usually recommended dose of furosemide is 40 to 200 mg/day Doses of furosemide of 980 mg/day (7) and 1800 mg/day (5 14) have been used on occasion and no toxic effects have been noted In three of our four cases (cases 1 3 and 4) 360 600 and 360 mg respectively of furosemide per day were required before a diuretic response occurred In case 2 however there was no diuretic response with doses up to 1200 mg/day The reason for this peculiar resistance of some patients to the administration of furosemide is not readily apparent A poor diuretic response may be expected when there is a marked decrease in glomerular filtration rate (10) This is particularly the case when the creatinine clearance falls below 5 ml/min In our cases however the creatinine clearances were well above this The sodium reabsorbing tendency of the tubules is probably the major factor determining response to the diuretic but factors governing this excessive reabsorption are obscure Furosemide in the usually administered doses blocks sodium reabsorption primarily in the ascending limb of the loop of Henle (2 6 16 17) In doses higher than ordinarily used it may also reduce sodium reabsorption in the proximal tubule (13) Possibly it is this combined action on the two sites that accounts for the diuresis when higher doses are used

Combinations of furosemide with other diuretics may produce a diuretic response equal to or at times greater than their individual responses (3 5 14) In case 2 no diuresis occurred with 800 mg or 1200 mg of furosemide but the addition of a thiazide produced a profound diuresis In case 3 a moderate diuresis occurred with 600 mg and 800 mg furosemide daily but the addition of spironolactone increased it markedly A further increase was noted when a thiazide was added to the program

In no patient was a reduction in renal function noted as judged by BUN serum creatinine and creatinine clearance In case 4 indeed a marked improvement in these parameters occurred The reason for this is not entirely clear It is known that furosemide increases renal blood flow and glomerular filtration (10 11 19) It has been suggested that this is accomplished by an inhibition of renin release from the juxtaglomerular apparatus (1) The diuresis itself may improve renal function by reducing renal interstitial edema thus alleviating tubular obstruction Preliminary investigations suggest that the drug may play a useful role in the prevention and treatment of acute renal failure (1)

Toxicological evaluation of furosemide in animals has shown it to have a wide margin of safety (18) In our patients doses up to 1200 mg/day produced no toxic side effects No reports of bone marrow aplasia or liver toxicity have been reported nor did these side effects occur in our patients

Thrombocytopenia skin rash anorexia and diarrhea occasionally seen by others were not noted in our patients Isolated reports exist of abnormal glucose tolerance tests precipitated by furosemide (8) No changes in fasting blood sugar were noted in our patients and there was no change in the insulin requirements of case 1 who had diabetes The serum uric acid level not infrequently rises in patients taking furosemide but attacks of gout are exceedingly uncommon (5 14) The serum uric acid concentrations did not change in our patients and attacks of gout did not occur

Hypokaliemia frequently seen with the administration of furosemide (5 14) was seen in three cases but was extreme only in one (case 4) The loss of potasium and hydrogen is probably not caused by a direct effect of furosemide

on the tubules but by secondary aldosteronism This complication may be partially or completely prevented by reducing the speed of diuresis This is most effectively done by reduction of the dose or administration of the drug every second or third day Potassium chloride supplements administration of spironolactone or triamterene and the administration of ammonium chloride or arginine monochloride (rarely necessary) may all help in the prevention and treatment of this complication The chloride ion is as important as the potassium ion in the correction of the alkalosis (12)

Intravascular fluid depletion with postural hypotension decreases renal function and shock may be seen if diuresis is excessive or rapid One of our cases (case 4) developed mild postural hypotension but this disappeared spontaneously within a few days The complication can frequently be prevented if the diuresis is not allowed to become excessive

Occasionally patients will be seen who respond initially and later become resistant to the drug In these patients correction of the hypokaliemic alkalosis may improve the diuretic response Drug resistance may also be countered by administering the drug every second or third day instead of daily Finally increasing the dose of furosemide or adding other diuretics may again produce a diuresis Three of our patients (cases 1 2 and 4) actually required progressively less furosemide to remain free of edema

Some workers (3) have found the diuresis produced by divided doses of furosemide to be greater than that produced by a single daily dose Using both regimens we have found the diuretic response to be quite similar A single morning dose however has the advantage of producing a diuresis during the day thus avoiding troublesome nocturia

In our patients combinations of all the usual diuretics including furosemide in doses up to 200 mg/day ACTH injections steroids azathioprine and intravenous albumin all failed to promote a diuresis Scarification and peritoneal dialysis were resorted to in two patients to control edema However three patients were relieved of their edema by merely raising the dose of furosemide and the fourth by a combination of furosemide with a thiazide This suggests that in the patient with nephrotic edema who is resistant to all diuretics including furosemide in the usually administered dose the dose of furosemide should be gradually increased (at a rate of 80–120 mg/day) until a diuretic response occurs Doses up to 1800 mg/day may be needed (5 14)

Another approach is to add other diuretic agents to the program when 200 mg of furosemide per day have failed to produce a diuresis Spironolactone or triamterene would seem to be the agents of first choice because unlike thiazides mercurials and carbonic anhydrase inhibitors they reduce the loss of both hydrogen and potassium in the urine If no diuretic response occurs these other diuretic agents may then be added If the edema still proves resistant the dose of furosemide may then be increased further

REFERENCES

1 Cantarovich F & Locatelli A Personal communication

2 Deetjen P Micropuncture studies on site and mode of diuretic action of furosemide Ann N Y Acad Sci 139 408 1966

3 Dettli L & Spring P Therapy with combinations of diuretic agents comparative studies Ann N Y Acad Sci 139 471 1966

4 Kleinfelder H Experimental investigations and clinical trials of furosemide a new diuretic Germ. med Mth 8 459 1963

5 Laragh J H Cannon P J Stason W B & Heinemann H O Physiological and clinical observations on furosemide and ethacrynic acid Ann N Y Acad. Sci 139 453 1966

6 Malnic F Vieira F L & Enokibara H Effect of furosemide on chloride and water excretion in single nephrons of the kidney of the rat Nature (Lond) 208 80 1965

7 McKenzie I F C Fairley K F & Baird C W A clinical trial of furosemide (Lasix) Med J Aust. 1 819 1966

8 Mustala, O & Toivonen S Comparison of the diabetogenic effects of chlorothiazide and furosemide Ann Med intern Fenn 54 75 1965

9 Muth R Diuretic response to furosemide in the presence of renal insufficiency J Amer med Ass. 195 1066 1966

10 Reubi F C Clinical use of furosemide Ann. N Y Acad Sci 139 433 1966

11 Schirmeister J & Willmann H Über die Harnsäure- und andere Clearances nach intravenöser Gabe von Fursemide beim Menschen Klin Wschr 4 6 3 1964

12 Schwartz, W B Pathogenesis and replacement of diuretic induced potassium and chloride loss Ann N Y Acad Sci 139 506 1966

13 Seldin D W Eknoyan G Suki, W N & Rector F C Jr Localization of diuretic action from pattern of water and electrolyte excretion Ann N Y Acad Sci 139 328 1966

14 Stason W B Cannon P J Heinemann H O & Laragh J H Furosemide a clinical evaluation of its diuretic action Circulation 34 910 1966
15 Stokes W & Nunn L C A A new effective diuretic Lasix Brit Med J 2 910 1964
16 Suki W Rector F C., Jr & Seldin D W The site of action of furosemide and other sulfonamide diuretics in the dog J Clin Invest 44 1458 1965
17 Suzuki F Klutsch K & Heidland A Stop-flow Untersuchungen zum Wirkenmechanismus von Fursemid Klin. Wschr 4. 569 1964
18 Thoms R K Springman F R & Wilson H E A toxicological evaluation of furosemide a new diuretic agent Farmaco Ed prat 19 544 1964
19 Thurau K Influence of sodium concentration at macula densa cells on tubular sodium load Ann N Y Acad Sci 139 388 1966

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AN EVALUATION OF DC SHOCK TREATMENT OF ATRIAL ARRHYTHMIAS

Immediate Results and Complications in 437 Patients with Long term Results in the First 290 of these

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Abstract A total of 1098 DC shocks were given in 57– attempts at reversion of permanent atrial arrhythmias in 437 patients Of these 348 (80%) reverted to sinus rhythm during the first attempt.

Complications of the DC shock itself were infrequent Ventricular arrhythmias and bradycardia occurred usually of short duration. Eight patients developed ventricular fibrillation and in one instance the episode was dramatic Digitalis toxicity disposed patients to some of the ventricular arrhythmias. No deaths considered due to the DC shock were observed.

Thirteen patients (3%) developed an embolic episode 6 hours–6 days after reversion. Only two of these were among the 2 8 patients who were on anticoagulant therapy as against 11 among the 209 who were not Quinidine syncope caused by ventricular fibrillation was observed in eight patients

The 290 patients first treated were followed up after the first attempt at reversion until relapse of the atrial arrhythmia or for a minimum of two years After this period 23% of the patients treated were in sinus rhythm The sinus rhythm attained was most stable in the patients with operated mitral stenosis atrial septal defect and thyrotoxicosis that received treatment The stability gradually decreased with increasing duration of the preceding arrhythmia but was little influenced by the heart size and even less by functional classification Good long term results were obtained in individual patients with all etiological and anatomical types of heart disease

Simple criteria can hardly be given for selection of patients who will benefit from reversion of atrial arrhythmia and careful evaluation of the individual patient is required

Discovery of a new promising therapy is nowadays very soon followed by a wave of medical enthusiasm Since the introduction of DC shock treatment of cardiac arrhythmias in 1962 (9) a vast literature on this subject has already been published (8 11 12 15 20)

Undoubtedly DC shock represents a very important advance in cardiological therapy But there is as already pointed out by Oram equally no doubt that it has been used too enthusiastically (13) A more critical assessment of the indications for and value of this treatment is now gradually being established

The purpose of this study is

1 to present the immediate results and complications of DC shock therapy in 437 patients with atrial arrhythmias

2 to present the long term results in the first 290 of these patients who have all been followed up either until relapse of their atrial arrhythmia occurred or for a minimum of 24 months

3 to see if more strict criteria can be established on the basis of our results and experiences for the selection of patients who will benefit from this treatment

During these first years of the trial our aim has been to obtain as extensive experience as possible Accordingly our indications have been most extensive we have tried DC shock in all patients admitted to our department with permanent atrial arrhythmia Our only contraindication was the presence of total A V block or an imminent cardiac operation In the last case attempts to obtain electrical reversion were postponed until after surgery

MATERIAL

A few remarks concerning the selection of the patients are appropriate Rikshospitalet, the University Hospital admits mainly patients referred from other hospitals all over the country It has the only fully developed cardiac surgical center in Norway As our department is working in close cooperation with this center we admit for hemodynamic studies and cardiological evaluation a much larger number of patients with serious acquired,

valvular diseases and congenital heart diseases, and a smaller number of patients with ischemic heart disease particularly acute myocardial infarction than is found in an unselected series of patients from our population. A considerable number of the patients with acquired valvular disease are at an advanced stage of their illness

In this study are included all the patients admitted to the department with permanent atrial fibrillation atrial flutter and atrial tachycardia with block during the period from March 1 1964 to November 1 1967 During this period DC shock therapy was applied to all these patients, if not prevented by the presence of one of the two contraindications mentioned above Thus a total of 1098 DC shocks were given in the course of 572 attempts to produce electrical reversion of atrial arrhythmia in 437 patients In 454 of these attempts the atrial arrhythmia was reverted to sinus rhythm. In 112 of the 437 patients repeated attempts to obtain reversion were carried out It should be stressed however that the data, which have been carefully analysed in this study and which will be presented are the immediate results of the first 437 attempts in respect of the total number of 437 patients, and the long term results of the *first* 290 attempts in the first 290 of these patients who were all followed up at least until relapse of the atrial arrhythmia or for a minimal period of two years

METHODS

All our patients had been digitalised with digitoxin This drug was omitted during the last 3–5 days before the procedure in all but the first 1.6 patients whose digitoxin was not discontinued

In 347 of the patients quinidine was given usually in daily doses of 1.2 g. This treatment was started 3 5 days before the DC shock treatment, to determine the patient s tolerance and serum quinidine concentrations before reversion of the atrial arrhythmia was attempted (In the first 93 patients quinidine was started either on the day or on the day before DC shock therapy was given) If sinus rhythm was not attained quinidine was stopped In all other cases it was continued indefinitely or until relapse of the atrial arrhythmia occurred except in a few patients where atrial fibrillation was due to a temporary cause (thyrotoxicosis operation for atrial septal defect) Only in 17 patients did quinidine intolerance preclude the use of this drug. Four of these *patients were treated with quinine in daily doses of 1 0 g* In the remaining 13 cases DC shock was given without any particular drug therapy to maintain sinus rhythm

In 73 patients a β adrenergic blocking agent (H 56 28) was tried as a matter of routine before and after electrical reversion was carried out These patients are not included in the follow up material presented in this study The result of this treatment will be published later (1)

In 228 patients, who were on long term anticoagulant therapy before admission to the department, this treatment was continued In 209 patients, who were not on long term anticoagulant therapy anticoagulants were not given in connection with the attempt to obtain electrical reversion (7)

The DC shock therapy was applied in a fasting state under general anesthesia, with a short acting barbiturate (thiopental sodium) Pethidine (meperidine) 50 mg administered subcutaneously was given as premedication 1 hour before operation

A 12 lead ECG was recorded immediately before the procedure A Corbin Farnsworth synchronized DC defibrillator with large-diameter electrodes, was used In approximately $^1/$ of the attempts anterior electrodes were applied in the apical basal position In the remaining $^2/_3$ the anterior posterior placement was used

If sinus rhythm was not established after the first shock of 100 ws and no signs of ventricular irritability (multiple ventricular premature beats or ventricular rhythm) developed further shocks were administered increasing the energy level to 200 300 and a maximum of 400 ws More than four shocks were not given during a single attempt. All the patients were ventilated with oxygen before and after each shock

After the procedure the patients were closely observed clinically and by means of an oscilloscope and ECG were repeatedly recorded until the patients were awake or for 5–10 minutes after the last shock was given They were then transferred to the ward and further closely observed by the attending nurse A new ECG was recorded 2–3 hours after the procedure The treatment with digitalis was resumed for all patients Quinidine (or H 56/28) was continued for all patients who were sent back to the ward in sinus rhythm or with A V nodal rhythm Blood quinidine levels were determined on the day of the procedure and daily for the followin few days

A patient was regarded as having undergone reversion if sinus rhythm was attained and persisted until the patient was returned to the ward that is for at least 5 10 minutes or if the DC shock therapy resulted in A V nodal rhythm which after some minutes or hours changed into sinus rhythm Patients who had only transient sinus rhythm lasting for only seconds or a few minutes, were not regarded as having undergone reversion

Before beginning the study special forms were prepared for recording the appropriate data and the follow up *observations for all the patients*

RESULTS

Table I gives a survey of the total material and the follow up material and the number and percentage of patients who reverted to sinus rhythm Age and sex distribution and the primary results are almost the same in the follow up material as in the total material Among the 437 patients treated with DC shock 348 (80%) primarily reverted to sinus rhythm

It should be mention d in this connection that among the 409 patients who were given quinidine before electrical reversion was attempted 62 (15%) reverted to sinus rhythm by means of th

Table I *Material*

Sex	Age (y) Mean	Range	No of pats	Reverted to sinus rhythm No	Per cent
Total material					
Male	51 6	10–77	197	152	77
Female	51 3	18–81	240	196	82
Total	51 4	10–81	437	348	80
Follow-up material					
Male	51 1	10–77	133	102	77
Female	50 8	18–81	157	133	85
Total	50 9	10 81	290	235	81

th rapy alone Consequently DC shock was not given and these patients are not included in the present material

The overall long term results in the follow up material are seen on the basal lin of Tables II–VII and in Fig 1 *Among the total number of* 290 patients treated with DC shock 235 (81°) reverted to sinus rhythm After 1 month 144 patients (50) *after 3 months 120 (41°) after* 6 months 104 (36°) after 12 months 83 (29°₀) and after 24 months 67 (23) of the patients *treated* were in sinus rhythm It should be pointed out that the percentages are calculated from the total numb r of patients treated (not patients reverted) These *results* are *similar to those* reported by other investigators (4 5 6 7 11 14 20 21)

Table II shows the primary results in the total material and the primary and long term results in the follow up material in relation to the type *of atrial arrhythmias treated* The mat°rial includes patients with permanent atrial fibrillation as well as atrial flutter and atrial tachycardia with block *We wish to point out that the distinction* between these three types of arrhythmia is not always very strict as transition from on° type to *another is frequently seen in the same* patient We have classified our patients according to the arrhythmia recorded on the ECG immediately before DC shock was given

It is remarkable that atrial flutter which is one of the most difficult arrhythmias to terminate with *quinidine and other drugs proves* to be easily reverted with DC shock It is evident from the table that both immediate and long term results in our material are somewhat *better for patients* with atrial flutter than for those with atrial fibrillation This is in agreement with results re*ported by other authors* (8 20)

The results observed in atrial tachycardia with block are also encouraging but the figures are small *It should be borne in mind that DC shock* is contraindicated in this condition until digitalis toxicity has been excluded as the cause

Table III shows the results in relation to etiology Our material is dominated by patients with rheumatic valvular disease who represent 274 of the 437 patients i e almost /₃ of the cases When considering the subgroups of patients with rheumatic etiology it is evident that the best primary and long term results were obtained in patients with operated mitral stenosis Of these 89 ₀ primarily reverted to sinus rhythm and in the follow up material 20 of the 56 patients who had been treated were still in sinus rhythm

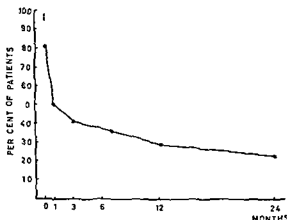


Fig 1 Percentage of the ²90 patients first treated who were in sinus rhythm after different intervals of time

Table II *Results in relation to type of atrial arrhythmia*

Type of arrhythmia	Total material: Total no of pats.	Reverted to SR: No of pats	Reverted to SR: Per cent	Follow up material: Total no of pats.	No of pats reverted to SR	Number of patients in SR after more than: 1 mo	3 mo	6 mo	12 mo	4 mo.
Atrial fibrillation	372	290	78	251	200	115	98	8[illegible]	65	51
Atrial flutter	46	41	89	[illegible]9	26	21	17	17	14	12
Atrial tachycardia with block	19	17	89	10	9	8	5	5	4	4
Total	437	348	80	290	235	144	1[illegible]0	104	83	67

SR = sinus rhythm.

more than two years after treatment The good results in our material in patients with operated mitral stenosis are not in agreement with results reported by some investigators (7 21) but are more similar to those published by others (11 14)

Much less satisfactory are the results particularly the long term results for the patients with mitral regurgitation and also for the more complicated cases in which mitral valvular disease is combined with other valvular lesions Of the 45 patients in both these categories in the follow up material treated with DC shock only about 10% (4 and 5 patients respectively) were in sinus rhythm after more than two years These results correspond with those obtained by other authors (8 11)

With regard to the non-rheumatic etiological categories listed in the table it is evident that the best immediate and follow up results were obtained in patients with atrial arrhythmia of thyrotoxic etiology It should be emphasized that electrical reversion was not attempted in these patients before they had been successfully treated for their hyperthyroidism The good results achieved in this etiological group are reported also by others (5)

The least satisfactory results were obtained in

Table III *Results in relation to etiology*

Etiology	Total material: Total no of pats.	Reverted to SR: No of pats.	Reverted to SR: Per cent	Follow up material: Total no of pats	No of pats. reverted to SR	Number of patients in SR after more than: 1 mo	3 mo	6 mo	12 mo	4 mo
Rheumatic										
MS	2	18	82	15	1[illegible]	6	5	4	3	3
MS op	84	75	89	56	50	33	30	8	[illegible]4	0
MR MR + MS	71	58	82	45	37	18	13	9	5	4
AS AR	16	13	81	13	11	6	4	4	3	3
M + other	77	56	73	45	36	[illegible]3	19	13	8	5
Constr per	4	3		4	3	0				
Total	274	[illegible]23	81	178	149	86	71	58	43	35
Art scl and/or hypert	64	47	73	40	31	4	19	17	15	11
Congenital	33	26	79	21	16	12	10	10	9	
Thyrotoxic	13	11	85	10	9	7	7	7	6	6
"Lone fibr"	27	19	70	22	15	8	7	7	5	-
Others	26	2[illegible]	85	19	15	7	6	5	5	4
Total	437	348	80	290	235	144	1[illegible]0	104	83	67

SR = sinus rhythm. MS = mitral stenosis. MS op = operated mitral stenosis. MR = mitral regurgitation. AS = aortic stenosis AR = aortic regurgitation. M + other = mitral and other valves affected Constr per = constrictive pericarditis.

Table IV *Results in relation to relative heart volume*

Relative heart volume (ml/m²)	Total material			Follow up material						
	Total no of pats	Reverted to SR		Total no of pats.	No of pats reverted to SR	Number of patients in SR after more than				
		No of pats	Per cent			1 mo	3 mo	6 mo	12 mo	24 mo
≤499	79	64	80	54	43	30	26	26	20	14
500–599	136	113	83	85	71	44	38	30	27	25
600–799	152	123	81	92	80	48	37	32	25	21
800–999	50	36	72	4	31	17	16	13	10	7
≥1000	17	11	65	14	9	5	3	3	1	0
Unknown	3	1		3	1	0				
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm.

the patients with "lone atrial fibrillation" without any other sign of heart disease The reversion rate in this group was 70% and only two of the 22 patients treated were in sinus rhythm after more than two years Hemodynamic studies have also shown that this category of patients benefits little from reversion of their atrial fibrillation (8)

In the group of patients with arteriosclerotic and/or hypertensive heart disease the primary results are less satisfactory but the follow up results are somewhat better than in the total group of patients with rheumatic valvular disease Similar findings have been published by other investigators (6) but on the whole the results are variable (7 11 21)

In Table IV the results are seen in connection with the relative heart volume as determined by roentgenological examination The size of the heart has little influence on the primary and long term results until pronounced enlargement is present It is only when the relative heart volume is increased above 800 ml/m that a considerable deterioration in the results is demonstrated More or less similar results have been reported by others (5 7 11 14)

The results in relation to the functional classification of the patients at the time of reversion (according to the New York Heart Association) are given in Table V Functional classes I and II are compared with III and IV The primary and the long term results are hardly better in the first group Morris et al (11) who classified their patients in the same way found a more impressive difference This may be due to a different selection of patients

Much more marked is the relation of the results to the duration of the preceding arrhythmia as demonstrated in Table VI The reversion rate decreases gradually with increasing duration of preceding arrhythmia particularly when it has been present for more than two years This influence is even more clearly shown in the long term results Thus the stability of the attained sinus rhythm is also seen to decrease gradually with

Table V *Results in relation to functional classification*

Functional class	Total material			Follow up material						
	Total no of pats	Reverted to SR		Total no of pats	No of pats reverted to SR	Number of patients in SR after more than				
		No of pats	Per cent			1 mo	3 mo	6 mo	12 mo	24 mo
I and II	312	255	8	195	162	103	87	75	60	45
III and IV	125	93	74	95	73	41	33	29	23	22
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm.

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AN EVALUATION OF DC SHOCK TREATMENT OF ATRIAL ARRHYTHMIAS

Immediate Results and Complications in 437 Patients with Long term Results in the First 290 of these

Christopher Bjerkelund and Otto M Orning

From Medical Department B University Hospital Rikshospitalet Oslo Norway

Abstract A total of 1098 DC shocks were given in 572 attempts at reversion of permanent atrial arrhythmias in 437 patient Of these 348 (80%) reverted to sinus rhythm durin the first attempt.

Compl cations of the DC shock itself were infrequent Ventricular arrhythmias and bradycardia occurred usually of short duration. Eight patients developed ventricular fibrillation and in one instance the episode was dramatic Digitalis toxicity disposed patients to some of the ventric ular arrhythmias No deaths considered due to the DC shock were observed.

Thirteen patients (3) developed an embolic episode 6 hours–6 days after reversion. Only two of these were among the 2?8 pa ients who were on anticoagulant therapy as against 11 among the 209 who we e not. Quinidine syncope caused by ventricular fibrillation was observed in eight patients

The 290 patients first treated were followed up after the first attempt at reversion until relapse of the atrial arrhythmia or for a m nimum of two years After this period 3% of the patients treated were in sinus rhythm. The sinus rhythm atta ned was most stable in the pa tients with operated m tral stenosis atrial septal defect and thyrotoxicosis that received treatment The stability gradually decreased with increasing duration of the preceding arrhythmia but was little influenced by the heart size and even less by functional classification Good long term results were obtained in individual patients with all etiological and anatomical types of heart disease

Simple criteria can hardly be given for selection of pa tients who will benefit from reversion of atrial arrhyth mia and careful evaluation of the individual patient is required

Discovery of a new promising therapy is nowa days very soon followed by a wave of medical enthusiasm Since the introduction of DC shock treatment of cardiac arrhythmias in 1962 (9) a vast literature on this subject has already been published (8 11 12 1? 20)

Undoubtedly DC shock represents a very im portant advance in cardiological therapy But there is as already pointed out by Oram [illegible] doubt that it has been used too [illegible] (13) A more critical assessment of the [illegible] tions for and value of this treatment [illegible] gradually being established

The purpose of this study is

1 to present the immediate results [illegible] plications of DC shock therapy in 437 [illegible] with atrial arrhythmias

2 to present the long term results [illegible] 290 of these patients who have all been [illegible] up either until relapse of their atrial [illegible] occurred or for a minimum of 24 months

3 to see if more strict criteria can be [illegible] on the basis of our results and exper[illegible] the election of patients who will be [illegible] this treatment

During these first years of the trial [illegible] has been to obtain as extensive exper[illegible] possible Accordingly our indications ha[illegible] mo t extensive we have tried DC shock [illegible] patients adm tted to our d partment w[illegible] manent atrial arrhythmia Our only contra[illegible] tion was the presence of total A V block [illegible] imminent cardiac operation In the last case [illegible] tempts to obtain electrical reversion w re [illegible] poned until after surgery

MATERIAL

A few remarks concerning the selection of the [illegible] are appropriate Rikshosp talet the University H[illegible] admits mainly pat ents referred from other hosp[illegible] over the country It has the only fully developed [illegible] surgical center in Norway As our department is [illegible] in close cooperation with this center we admit [illegible] hemodynamic studies and cardiological [illegible] much larger number of patients with

val ular diseases and congenital heart diseases and a smaller number of patients with ischemic heart disease particularly acute myocardial infarction than is found in an unselected series of patients from our population A considerable number of the patients with acquired val ular disease are at an advanced stage of their illness

In this study are included all the patients admitted to the department wi h permanent atrial fibrillation, atrial flutter and atrial tachycardia with block during the period from March 1 1964 to November 1 1967 During this period DC shock therapy was applied to all these patients if not prevented by the presence of one of the two contraindications mentioned above Thus, a total of 1098 DC shocks were given in the course of 57? attempts to produce electrical reversion of atrial arrhythmia in 437 patients. In 454 of these attempts the atrial arrhythmia was reverted to sinus rhythm In 112 of the 437 patients repeated attempts to obtain reversion were carried out It should be stressed however that the data which have been carefully analysed in this study and which will be presented are the immediate results of the fi st 437 attempts in respect of the total number of 437 patients, and the long term results of the *first* 290 attempts in the first ?90 of these patients who were all followed up at least until relapse of the atrial arrhythmia or for a m nimal period of two years.

METHODS

All our patients had been digitalised with digitoxin This drug was omitted during the last 3–5 days before the procedure in all but the first 1 6 patients whose d gitoxin was not discon inued

In 347 of the pat ents quinidine was given usually in daily doses of 1 ? g This trea ment was started 3 5 days before the DC shock treatment, to determine the pa tient s tolerance and serum quinidine concentrations be fore reversion of the atrial arrhythmia was attempted (In the first 93 patients quinidine was started either on the day or on the day before DC shock therapy was gi en) If sinus rhythm was not attained quinidine was stopped In all other cases it was continu d indef nitely or until relapse of the atrial arrhythmia occurred except in a few patients where atrial fibrillation was due to a temporary cause (thyrotoxicosis, operation for atrial septal defect) Only in 17 patients did quinidine in tolerance preclude the use of this drug. Four of these patients were treated with quinine in daily doses of 1 0 g In the remaining 13 ases DC shock was given without any particular drug therapy to maintain sinus rhythm

In 73 patients a β adrenerg c blocking agent (H 56 28) was tried as a matter of routine before and after el c trical reversion was carried out These patients are not included in the follow up material presented in this study The result of this treatment will be published later (1)

In 228 patients, who were on long-term anticoagulant therapy before admission to the department, this treat ment was continued In 209 patients, who were not on long term anticoagulant therapy anticoagulants were not given in connection with the attempt to obtain electrical reversion (7)

The DC shock therapy was applied in a fasting state under general anesthesia, with a short acting barbiturate (thiopental sodium) Pethidine (meperidine) 50 mg ad ministered subcutaneously was given as premedi ation 1 hour before operation

A 12 lead ECG was recorded immediately before the procedure A Corbin Farnsworth synchronized DC de fibrillator with large-diameter electrodes, was used In approximately ³/ of the attempts, anterior electrodes were applied in the apical basal position In the remaining ¹/₄ the anterior posterior placement was used.

If sinus rhythm was not established after the first shock of 100 ws, and no signs of ventricular irritability (multiple ventricular premature beats or ventricular rhythm) developed further shocks were administered increasing the energy level to 200 300 and a maximum of 400 ws More than four shocks were not given during a single attempt. All the patients were ventilated with oxygen before and after each shock

After the procedure the patients were closely observed clinically and by means of an oscilloscope and ECG were repeatedly recorded until the patients were awake or for 5–10 minutes after the last shock was given They were then transferred to the ward and further closely observed by the attending nurse A new ECG was re corded 2–3 hours after the procedure The treatment with digitalis was resumed for all patients Quinidine (or H 56/ 8) was continued for all patients who were sent back to the ward in sinus rhythm or with A V nodal rhythm Blood quinidine levels were determined on the day of the procedure and daily for the followin few days

A patient was regarded as having undergone reversion if sinus rhythm was attained and persisted until the pa tient was returned to the ward that is for at lea t 5–10 minutes or if the DC shock therapy resulted in A V nodal rhythm which after some minutes or hours, changed in o sinus rhythm Patients who had *only transient* sinus rhythm lasting for only seconds *or a few* minutes, were not regarded as having underg ne rever sion

Before beginning the study special forms were prepared for recording the appropriate data and the follow up observations for all the patients

RESULTS

Table I gives a survey of the total material and the follow up material and the number and per centage of patients who reverted to sinus rhythm Age and sex distribution and the primary results are almost the same in the follow up material as in the total material Among the 437 patients treated with DC shock 348 (80%) primarily re verted to sinus rhythm

It should be mention d in this connection that, among the 409 patients who were given quin d e before electrical reversion was attempted 62 (15%) reverted to sinus rhythm by means of th s

Table I *Material*

Sex	Age (y) Mean	Range	No of pats	Reverted to sinus rhythm No	Per cent
Total material					
Male	51 6	10–77	197	152	77
Female	51 3	18–81	240	196	82
Total	51 4	10–81	437	348	80
Follow up material					
Male	51 1	10–77	133	10?	77
Female	50 8	18–81	157	133	85
Total	50 9	10–81	290	235	81

th rapy alone Consequently DC shock was not given and these patients are not included in the present material

The overall long term results in the follow up material are seen on the basal line of Tables II–VII and in Fig 1 Among the total number of 290 patients treated with DC shock 235 (81%) reverted to sinus rhythm After 1 month 144 patients (50%) after 3 months 120 (41%) after 6 months 104 (36%) after 12 months 83 (29%) and after 24 months 67 (23%) of the patients *treated* were in sinus rhythm It should be pointed out that the percentages are calculated from the total numb r of patients treated (not patients reverted) These results are similar to those reported by other investigators (4 5 6 7 11 14 20 21)

Table II shows the primary results in the total material and the primary and long term results in the follow up material in relation to the type of atrial arrhythmias treated The material includes patients with permanent atrial fibrillation as well as atrial flutter and atrial tachycardia with block We wish to point out that the distinction between these three types of arrhythmia is not always very strict as transition from one type to another is frequently seen in the same patient We have classified our patients according to the arrhythmia recorded on the ECG immediately before DC shock was given

It is remarkable that atrial flutter which is one of the most difficult arrhythmias to terminate with quinidine and other drugs proves to be easily reverted with DC shock *It is evident from* the table that both immediate and long term results in our material are somewhat better for patients with atrial flutter than for those with atrial fibrillation This is in agreement with results reported by other authors (8 20)

The results observed in atrial tachycardia with block are also encouraging but the figures are small It should be borne in mind that DC shock is contraindicated in this condition until digitalis toxicity has been excluded as the cause

Table III shows the results in relation to etiology Our material is dominated by patients with rheumatic valvular disease who represent 274 of the 437 patients i e almost /3 of the cases When considering the subgroups of patients with rheumatic etiology it is evident that the best primary and long term results were obtained in patients with operated mitral stenosis Of these 89 primarily reverted to sinus rhythm and in the follow up material 20 of the 56 patients who had been treated were still in sinus rhythm

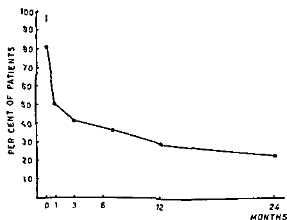


Fig 1 Percentage of the ?90 patients first treated who were in sinus rhythm after different intervals of time

Table II *Results in relation to type of atrial arrhythmia*

Type of arrhythmia	Total material: Total no of pats.	Reverted to SR: No of pats	Reverted to SR: Per cent	Follow up material: Total no of pats.	No of pats reverted to SR	Number of patients in SR after more than: 1 mo	3 mo	6 mo	12 mo	24 mo
Atrial fibrillation	372	290	78	251	200	115	98	8	65	51
Atrial flutter	46	41	89	29	26	21	17	17	14	1
Atrial tachy *cardia with block*	*19*	*17*	89	*10*	*9*	*8*	*5*	*5*	*4*	*4*
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm

more than two years after treatment The good results in our material in patients with operated mitral stenosis are not in agreement with results reported by some investigators (7 21) but are more similar to those published by others (11 14)

Much less satisfactory are the results partic ularly th long term results for the patients with mitral regurgitation and also for the more com plicated cases in which mitral valvular disease is combined with other valvular lesions Of the 45 patients in both these categories in the follow up material treated with DC shock only about 10% (4 and 5 patients respectively) were in sinus rhythm after more than two years These results cor respond with those obtained by other authors (8 11)

With regard to the non rheumatic etiological categories listed in the tabl it is evident that the best immediate and follow up results were ob tained in patients with atrial arrhythmia of thyro toxic etiology It should be emphasized that elec trical reversion was not attempted in these patients before they had been successfully treated for their hyperthyroidism The good results achieved in this etiological group are reported also by oth rs (5)

Th least satisfactory results were obtained in

Table III *Results in relation to etiology*

Etiology	Total material: Total no of pats	Reverted to SR: No of pats.	Reverted to SR: Per cent	Follow up material: Total no of pats	No of pats reverted to SR	Number of patients in SR after more than: 1 mo	3 mo	6 mo	12 mo	24 mo
Rheumatic										
MS	2	18	82	15	12	6	5	4	3	3
MS op	84	75	89	56	50	33	30	28	4	0
MR MR MS	1	58	82	45	37	18	13	9	5	4
AS AR	16	13	81	13	11	6	4	4	3	3
M + other	7	56	73	45	36	23	19	13	8	5
Constr per	4	3		4	3	0				
Total	274	223	81	178	149	86	71	58	43	35
Art. scl. and or hypert	64	47	73	40	31	24	19	17	15	13
Congenital	33	26	79	21	16	12	10	10	9	7
Thyrotoxic	13	11	85	10	9	7	7	7	6	6
"Lone fibr"	27	19	70	22	15	8	7	7	5	
Others	26	22	85	19	15	7	6	5	5	4
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm. MS = mitral stenosis. MS op = operated mitral stenosis. MR = mitral regurgitation AS = aortic stenosis. AR = aortic regurgitation M + other = mitral and other valves affected Constr per = constrictive pericarditis.

Table IV *Results in relation to relative heart volume*

Relative heart volume (ml/m²)	Total material			Follow up material						
	Total no of pats	Reverted to SR		Total no of pats	No of pats reverted to SR	Number of patients in SR after more than				
		No of pats	Per cent			1 mo	3 mo	6 mo	12 mo	24 mo
≤499	79	64	80	54	43	30	26	6	20	14
500–599	136	113	83	85	71	44	38	30	27	25
600–799	152	123	81	92	80	48	37	32	25	21
800–999	50	36	72	42	31	17	16	13	10	7
≥1000	17	11	65	14	9	5	3	3	1	0
Unknown	3	1		3	1	0				
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm.

the patients with lone atrial fibrillation without any other sign of heart disease. The reversion rate in this group was 70% and only two of the 22 patients treated were in sinus rhythm after more than two years. Hemodynamic studies have also shown that this category of patients benefits little from reversion of their atrial fibrillation (8).

In the group of patients with arteriosclerotic and/or hypertensive heart disease the primary results are less satisfactory but the follow up results are somewhat better than in the total group of patients with rheumatic valvular disease. Similar findings have been published by other investigators (6) but on the whole the results are variable (7, 11, 21).

In Table IV the results are seen in connection with the relative heart volume as determined by roentgenological examination. The size of the heart has little influence on the primary and long term results until pronounced enlargement is present. It is only when the relative heart volume is increased above 800 ml/m² that a considerable deterioration in the results is demonstrated. More or less similar results have been reported by others (5, 7, 11, 14).

The results in relation to the functional classification of the patients at the time of reversion (according to the New York Heart Association) are given in Table V. Functional classes I and II are compared with III and IV. The primary and the long term results are hardly better in the first group. Morris et al (11) who classified their patients in the same way found a more impressive difference. This may be due to a different selection of patients.

Much more marked is the relation of the results to the duration of the preceding arrhythmia as demonstrated in Table VI. The reversion rate decreases gradually with increasing duration of preceding arrhythmia particularly when it has been present for more than two years. This influence is even more clearly shown in the long term results. Thus the stability of the attained sinus rhythm is also seen to decrease gradually with

Table V *Results in relation to functional classification*

Functional class	Total material			Follow up material						
	Total no of pats.	Reverted to SR		Total no of pats	No of pats. reverted to SR	Number of patients in SR after more than				
		No of pats	Per cent			1 mo	3 mo	6 mo	12 mo	24 mo
I and II	312	55	82	195	162	103	87	75	60	45
III and IV	125	93	74	95	73	41	33	29	23	22
Total	437	348	80	290	235	144	120	104	83	67

SR = sinus rhythm

Table VI *Results in relation to duration of preceding arrhythmia*

Duration of atrial arrhythmia (mo)	Total material			Follow up material						
	Total no of pats	Reverted to SR		Total no of pats	No of pats reverted to SR	Number of patients in SR after more than				
		No of pats	Per cent			1 mo	3 mo	6 mo	12 mo	24 mo
< 1	41	37	90	22	19	17	14	13	13	12
1– 5	93	85	91	50	47	33	29	28	23	19
6–23	91	80	88	63	58	39	37	33	24	20
24–59	77	60	78	60	47	29	22	18	16	12
> 60	135	86	64	95	64	26	18	12	7	4
Total	437	348	80	290	235	144	1[illegible]0	104	83	67

SR = sinus rhythm

increasing duration of the preceding arrhythmia This result is in good agreement with those reported by others (7 8 11 21) and also corresponds well with previous experience with quinidine induced reversion of atrial fibrillation (18 19) It should be remembered however that atrial fibrillation of long duration is primarily seen in patients with serious heart disease particularly rheumatic valvular disease at an advanced stage But exceptions to this rule especially lone fibrillation and atrial fibrillation in old age are also well known

In Table VII the long term results are presented in relation to the energy level required for the patients to revert to sinus rhythm It is clearly ...onstrated that early relapses are more fre... observed in patients who needed a high energy level for reversion In fact there is also a marked difference in the long term results between those patients who required 100 ws for reversion and those who needed 200 ws Fourty four % of the first group and only 20% of the second were in sinus rhythm after more than two years of observation Similar findings have been reported (7)

Among the 67 patients who retained sinus rhythm for more than 24 months 58 were still in sinus rhythm at their last control in the summer or autumn of 1967 They had then been observed for an average of 32 2 months per patient varying between 24 and 45 months

Of these 58 patients 28 had rheumatic valvular disease 13 arteriosclerotic heart disease seven operated atrial septal defect five thyrotoxicosis (treated) two primary myocardial disease one operated constrictive pericarditis and two lone atrial fibrillation" Among the 28 cases with rheumatic etiology 19 were cases of operated mitral stenosis two not operated mitral stenosis one mitral regurgitation three mitral and aortic valvular disease and three aortic valvular disease Thus it is evident that beneficial results of long duration can be obtained in patients with heart disease belonging to all etiological categories

Table VII *Follow up results in patients reverted to sinus rhythm in relation to energy level*

Energy level (ws)	No of pats reverted to SR	Number of patients remaining in SR after more than				
		1 mo	3 mo	6 mo	12 mo	24 mo
100	87	61	53	49	43	39
200	102	62	49	38	27	20
300	28	11	10	9	7	5
400	18	10	8	8	6	3
Total	235	144	120	104	83	67

SR = sinus rhythm.

Deaths During Observation Period

Nine patients died during the observation period i.e. either before relapse of their atrial arrhythmia had been diagnosed or before the end of the observation period

Three died in the department

A 30 year-old woman who had been admitted at the terminal stage of chronic myocarditis died of serious congestive heart failure 12 days after reversion of an atrial tachycardia with block and clearly without any causative relationship to the DC shock therapy

A 67 year old woman with aortic stenosis and regurgitation and a history of angina pectoris and fits of dizziness who was suffering from serious congestive heart failure and 12 days previously had had a cerebral embolus died suddenly during the night 12 hours after the procedure She was on quinidine Autopsy did not disclose signs of fresh emboli or any other cause of sudden death

A man aged 57 with advanced mitral stenosis and regurgitation relative heart volume of 840 ml/m and congestive failure died suddenly during the night 15 hours after reversion of his atrial fibrillation with DC shock He was on quinidine No autopsy

Moreover three patients died suddenly at home 5 14 and 16 days after the electrical reversion of their atrial fibrillation and clearly without any direct relation to this procedure They were all on quinidine No autopsy

Finally three patients died at home most probably of heart disease 3 10 and 25 months after the treatment with DC shock

For all patients who died at home information was obtained from their physician and/or from their relatives All the nine patients mentioned are included in the follow up material with an observation period from the reversion of their atrial arrhythmia until death

It should be emphasized that the figures given in this paper are the results of the *first* attempt at electrical reversion in each patient Our results are clearly less discouraging than those recently reported by Coelho et al (4) who found that only 5 9 % of their 219 *reverted* patients remained in sinus rhythm for more than 24 months Our figures cannot be compared with those reported by Morris et al (11) who presented the results of maintaining sinus rhythm with multiple reversions in the same patient By this means they succeeded in maintaining sinus rhythm in the 20th month in 53 % of their 108 patients

Repeated attempts at reversion were however also tried in our patients Thus in 112 of the patients 247 attempts were made varying between two and a maximum of ten attempts per patient The result of these trials will not be presented here It should be mentioned however, that in a few of these patients sinus rhythm was maintained for a considerably longer period after the second reversion than after the first

Three of the patients in the present material were treated with DC shock because atrial arrhythmia developed while they were being treated continuously with implanted P synchronous pacemakers In two of these patients the atrial arrhythmia was reverted to sinus rhythm judging from the P waves in the ECG but only in one patient was a synchronous pacemaker activity obtained One patient maintained normal atrial activity for more than 25 months The other two patients did not belong to the follow up material

Complications

Four categories of complications are of interest in connection with DC shock therapy

1 Complications related to the DC shock itself

2 Complications related to the reversion of the atrial arrhythmia

3 Complications related to the anesthesia

4 Complications related to the drug treatment given in connection with the attempt to obtain electrical reversion of the atrial arrhythmia

We will describe each of these categories separately

1 The most common complications of the DC shock itself are disturbances in heart rhythm and conduction in most cases of benign character and short duration Thus transient multiple ventricular premature beats frequently as bigeminal rhythm occurred in 120 of the 572 attempts made to obtain reversion in our patients Ventricular tachycardia occurred in 13 patients but was of only 2–20 seconds duration and reverted spontaneously to sinus rhythm or to the original atrial arrhythmia in all the patients

A more serious complication was ventricular fibrillation which occurred in eight patients In four of these the arrhythmia was transient lasting

for only 2–25 seconds and reverted to sinus rhythm or the original arrhythmia without any special treatment In the other four cases however the ventricular fibrillation was of longer duration namely 1 1¹/ 4 and 20 minutes respectively Ventilation with oxygen and external cardiac mas sage was immediat ly started and three of the patients were successfully defibrillated with one or two additional DC shocks

The most dramatic episode was observed in the last patient a 25 year old woman operated on three weeks previously for an atrial septal defect of primum type In connection with the operation she developed atrial flutter After the first DC shock of 100 ws ventricular fibrillation immedi ately developed and in spite of intubation and ventilation with oxygen external cardiac massage seven additional DC shocks and infusion of sodium bicarbonate persisted continuously until apparently spontaneously she reverted to sinus rhythm after 20 minutes She had recovered com pletely on the following day

Patients with advanced rheumatic valvular disease and large heart volumes had a special tend ency to develop ventricular arrhythmias Whether or not digitoxin was discontinued some days be fore DC shock therapy was given could not be shown to influence the incid n e of premature beats or ventricular arrhythmias after the proce dure In six of the patients in whom ventricular tachycardia or ventricular fibrillation d veloped ventricular premature beats were recorded im mediately before DC shock Premature beats especially if they increase in number after the first DC shock is a warning of impending ven tricular arrhythmia if another shock is given We believe that some of the cases of ventricular arrhythmia in our material develop d because we did not fully take account of this warning during the first period of our trial

Many patients temporarily developed nodal rhythm in response to DC shock treatment before a stable sinus rhythm was established In most cases the nodal rhythm lasted only some seconds or a few minutes but a few patients remained in this ectopic rhythm for hours or even a day or two and then reverted to atrial fibrillation

Five patients developed transient bradycardia following DC shock Complete atrioventricular block lasting for a maximum of 2 hours was the cause in three of these patients In the remaining two patients pronounced sinus bradycardia (18–30 beats per minute) was observed in one and persistent atrioventricular dissociation in the other Digitoxin was discontinued for 2–8 days before the treatment in all these five patients

T wave inversions in the ECG were not re corded in our patients Systematic enzyme studies were not made in connection with the DC shock therapy No deaths occurred in direct relation to the DC shock

2 Of the 437 patients in the present study 13 (3 %) experienced an embolic episode in connec tion with the treatment All the episodes were ob served in relation to successfull reversions Thus the incidence of embolic episodes in the 454 in stances of reversion was 2 9 % All the episodes were systemic emboli seven involved the arteries of the legs five the cerebral arteries and one the mesenteric artery Six of the embolic episodes oc curred in patients with rheumatic valvular disease and seven in the cases with non rheumatic etiology of these two were cases of lone atrial fibrilla tion

As already mentioned 228 of the patients were on long term anticoagulant therapy during the at tempt to achieve reversion of their atrial ar rhythmia and 209 were not given anticoagulants The number of embolic episodes in these groups were two and 11 respectively This difference is statistically significant A study is being published separately (2) of the efficacy of anticoagulant therapy in preventing embolism related to DC elec trical reversion of atrial arrhythmias

Pulmonary edema after reversion as observed by Resnekov and McDonald (16) did not develop in any of our patients

3 The only complication which was believed to be related to the anesthesia was a fall in the systolic blood pressure below 80 mm Hg which occurred in 1 patient The hypotension was suc cessfully treated with metaraminol and reversion was then carried out without further difficulties

4 Our policy regarding digitalis dosage in con nection with DC shock has already been men tioned The importance of digitalis as a contribut ing cause of the disturbances in rhythm observed after DC shock is difficult to evaluate During the first nine months of the trial we were impres sed by the high incidence of ventricular premature

beats and bigeminal rhythm after reversion to sinus rhythm Consequently in the patients sub sequently treated digitalis was discontinued 3–5 days before DC shock was given We believe that digitalis overdosage was a contributing cause to the development of ventricular tachycardia or ventricular fibrillation in six of our patients

During quinidine treatment before and after at tempts to obtain electrical reversion syncope was observed in eight of the first 230 patients in this series In four of these syncope occurred before reversion showing that DC countershock was not a contributing cause Five of the patients had only one attack of syncope but in three instances numerous attacks (15–50) were observed during 5–16 hours ECG monitoring in these patients showed that all the attacks of syncope were due to ventricular fibrillation This serious complication was heralded in the ECG by marked prolongation of the Q T interval ventricular premature beats bigeminy and short runs of ventricular tachy cardia All the attacks of syncope occurred within three days after quinidine therapy had been started In spite of the moderate doses of quinidine given (1 2 g/day) the determination of the serum quinidine concentration carried out in four of the patients showed relatively high values (6 3 6 8 8 0 14 7 mg/l) when the attacks were ob served Further details concerning these complica tions have been published previously (3) A more individual dosage of quinidine guided by the serum level of quinidine during the treatment has been found to be valuable and new attacks of quinidine syncope have not been observed in the last 117 patients treated with quinidine in this series

It should also be mentioned in this connection that the five patients who died suddenly between 12 hours and 16 days after reversion with DC shock were all being treated with maintenance doses of quinidine Therefore the possibility that quinidine may have been a contributing cause can not be excluded in these cases

As already mentioned 73 patients in this series were treated with a β blocking agent (H 56/28) in connection with the attempts to obtain electrical reversion of their atrial arrhythmias No serious complications were observed which could be re lated to this treatment The results of this thera peutic trial will be published separately (1)

DISCUSSION AND CONCLUSION

The purpose of a therapy is to benefit the pa tient Reversion of an atrial arrhythmia to sinus rhythm is theoretically considered to improve cardiac function counteract the tendency to con gestive heart failure and reduce the risk of systemic and pulmonary embolism This has been verified or supported by hemodynamic studies (10) and clinical observations

Assessment of the benefit to the individual pa tient would have to be based on a detailed and objective clinical and sociological evaluation of the patient s functional capacity during periods of atrial arrhythmia and after sinus rhythm had been well established We have not embarked upon this endeavour In a number of our patients it would have been difficult or even impossible to separate the effect of reversion from the effect of other therapeutic procedures particularly cardiac surgical corrections

Many patients did however describe consider able improvement and these statements were ac centuated by the fact that they felt a definite de terioration when relapse occurred and requested that another attempt be made to obtain reversion On the other hand a number of patients did not feel any difference between b ing in atrial fibrilla tion and being in sinus rhythm and did not re cognize the occurrence of a relapse of their ar rhythmia

From the results obtained in this series of pa tients it is evident that strict criteria cannot be established for the selection of patients who will benefit from reversion Some trends however seem to b clear and may be of help in the evalua tion of the indications

In relation to etiology (Table III) our best re sults were obtained in the groups of patients with operated mitral stenosis operated atrial septal defect and treated thyrotoxicosis However good long term results in individual patients were ob served in all etiological categories and anatomical types of heart disease No definite difference was found between the total group of rheumatic cases and that of non rheumatic cases

The size of the heart (Table IV) had little in fluence on the results except when excessive en largement was present The functional classifica tion of the patients (Table V) had also a very limited predictive value Much more striking was the relation of the results to the duration of the

preceding arrhythmia (Table VI) The stability of the sinus rhythm attained was found to gradually decrease with increasing duration of the preceding arrhythmia In patients who had had arrhythmia for more than two years the long term results were rather discouraging and where arrhythmia had been present for more than five years they were poor

In the evaluation of a therapy the incidence of complications is of decisive importance The observations made on this series of patients clearly show that the DC shock itself provokes surprisingly few serious complications This can be emphasized even more strongly when the serious cardiac condition of many of the patients treated is taken into consideration In spite of this no deaths considered due to the DC shock itself were observed in this relatively large number of patients In a few cases ventricular arrhythmias including ventricular fibrillation were observed but only one of these proved to be a really dramatic episode Most of the ventricular arrhythmias occurred in the first period of our trial and some of them were probably provoked because the warnings represented by premature beats before a shock was delivered were not fully taken into account

Two types of serious complications not directly related to DC shock were observed in the present series namely systemic embolism and quinidine induced ventricular fibrillation The embolic complications can probably be appreciably reduced when prophylactic anticoagulant therapy is given (2) The occurrence of quinidine induced complications was substantially reduced in the last part of this series when more individual dosage of quinidine was used guided by the serum quinidine level We wish to point out however that the definite value of quinidine in the prophylaxis against relapses of atrial fibrillation is not clearly established as no conclusive controlled clinical trials have ever been carried out

In conclusion we wish to emphasize that simple criteria for the indications for DC shock reversion of atrial arrhythmias can hardly be given When not only the DC shock itself but also the anesthesia and the different types of drugs involved in this treatment are taken into account it amounts to a considerable therapeutic maneuvre The indications should be carefully evaluated in each individual patient and the benefits which may eventually be achieved weighed against the inconveniences expences and risks If all these factors are taken into consideration before the treatment is given we believe that it will remain a valuable part of cardiological therapy

REFERENCES

1 Andersen A. & Hillestad L Personal communication.
2 Bjerkelund C & Orning O M The efficacy of anticoagulant therapy in preventing embolism related to DC electrical reversion of atrial fibrillation Amer J Cardiol In print
3 Bjerkelund C & Skåland K Kinidin som årsak til paroxystisk ventrikkelflimmer Nord Med 77 76 1967
4 Coelho E Pinto L S Luiz, A S Coelho F M Pereira A L. & Barreiros R. Long term results of conversion of atrial fibrillation by direct current countershock Cardiologia 50 147 1967
5 Halmos P B Direct current conversion of atrial fibrillation Brit Heart J 28 302 1966
6 Hurst J W Paulk E A., Jr Proctor H D & Schlant R. C Management of patients with atrial fibrillation Amer J Med 37 728 1964
7 Korsgren M Leskinen E., Peterhoff V Bradley E. & Varnauskas E Conversion of atrial arrhythmias with DC shock Acta med scand Suppl 431 1965
8 Lown B Electrical reversion of cardiac arrhythmias Brit Heart J 29 469 1967
9 Lown B Amarasingham R. & Neuman J New method for terminating cardiac arrhythmias Use of synchronized capacitor discharge J Amer med Ass. 182 548 196
10 Morris J J., Jr Entman M., North W C Kong Y & McIntosh H The changes in cardiac output with reversion of atrial fibrillation to sinus rhythm. Circulation 31 670 1965
11 Morris J J., Jr Peter R H & McIntosh H Electrical conversion of atrial fibrillation Immediate and long term results and selection of patients Ann intern Med 65 216 1966
12 Nachlas M M Bix H H Mower M M & Siedband M P Observations on defibrillators, defibrillation, and synchronized countershock Progr cardiovasc Dis. 9 64 1966
13 Oram S The treatment of cardiac arrhythmias by direct-current shock Proc roy Soc Med 60 371 1967
14 Oram S & Davies, J P H Further experiences of electrical conversion of atrial fibrillation to sinus rhythm Analysis of 100 patients Lancet 1 1?93 1964
15 Paulk E A & Hurst J W Clinical problems of cardioversion Amer Heart J 70 248 1965
16 Resnekov L & McDonald L Pulmonary edema following treatment of arrhythmias by direct-current shock Lancet 1 506 1965

17 — Complications in 220 patients with cardiac dysrhythmias treated by *phased direct current shock*, and indications for electroconversion. Brit Heart J 29 926 1967
18 Rokseth, R. Clinical considerations in *quinidine therapy of chronic auricular fibrillation A study of* 700 unselected patients with follow up Acta med scand. 174 171 1963
19 Rokseth, R. & Storstein O Quinidine therapy of chronic auricular fibrillation. Occurrence and mechanism of syncope Arch intern. Med 111 184 1963
20 Selzer A., Kelly J J Jr Johnson R. B & Kerth, W J *Immediate* and long term *results of* electrical conversion of arrhythmias. Progr cardiovasc Dis 9 90 1966
21 Wikland B Edhag O & Eliasch H Atrial fibrillation and flutter treated *with* synchronized DC shock A study on immediate and long term results Acta med scand 182 665 1967

Acta med scand Vol 184 pp 493–498 1968

RAPID SEMIQUANTITATIVE DETERMINATION OF CHOLINESTERASE ACTIVITY IN SERUM

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Abstract The Acholest test a simple semiquantitative method for determining plasma cholinesterase has been investigated. The results of this method agreed well with those obtained by the Warburg manometric method The test is therefore suitable as screening test and can be recommended for use prior to regular control of persons exposed to cholinesterase inhibitors mainly on organo phosphorus insecticide exposure

In recent years the determination of cholinesterase activity in plasma (butyrocholinesterase BuChE) and erythrocytes (acetylcholinesterase AChE) has become of great importance in the appraisal and treatment of poisoning by carbamates and organophosphorus insecticides Epidemiological studies of persons working with these insecticides have shown that many of them have a reduced cholinesterase activity without showing any clinical symptoms (8)

In 1959–1960 293 samples were taken for the determination of BuChE activity by the Warburg manometric method from 126 workers exposed to organophosphorus insecticides in an agricultural area in South Sweden (19) A good many of the 126 had reduced BuChE activity 56% had values less than 1 8 U ml⁻¹ 28% less than 1 35 U ml⁻¹ and 11% less than 0 3 U ml⁻¹ Of the 54 workers from whom more than one sample was taken during the spraying 18 (33%) had a reduction of BuChE activity of more than 50% of the highest analysed individual value Because the sample was taking during the exposure time the normal individual value was probably not obtained for everybody therefore the reduction of the BuChE activity of more than 50% in one third of the spraymen was rather too low

The epidemiological investigations and the annual occupational poisoning by preparations of the mentioned type emphasize the need for regular control with determination of cholinesterase activity of persons exposed to insecticides with cholinesterase inhibiting properties A control of this nature is arranged in some places (3 6)

The various quantitative methods for determination of cholinesterase activity among which the Warburg manometric method (2) has been predominant in Sweden is hardly suitable for routine control of a large number of exposed persons Therefore a simple rapid semiquantitative method for determination of cholinesterase activity safe enough for practical health control has been proposed as an obvious need

Determination of cholinesterase activity is valuable not only on poisoning by cholinesterase inhibitors in agriculture and horticulture but also on exposure to nerve gases at control of anticholinesterase therapy for patients with myasthenia gravis glaucome (14) and cancer (20) in the use of succinylcholine chloride for patients undergoing general anaesthesia (18 20) and for diagnosis of liver and kidney disorders (5) It is of practical importance on these occasions to have a rapid simple method that can be managed by untrained personnel and with limited laboratory resources

Semiquantitative methods for determination of cholinesterase activity are available commercially The Acholest test (Acholest paper obtainable from Österreichische Stickstoffwerke AG Linz, Austria) is a simple colorimetric method (17) that determines the BuChE activity in plasma or serum We have tested Acholest and compared the results with those from the determination of cholinesterase activity by the Warburg manometric method

METHOD AND TECHNIQUE

Samples were taken from workers with suspected exposure to organophosphorus insecticides, from hospital patients

Table I *Number and category of subjects number of samples and Acholest test findings*

	No samples	No persons	No persons with Acholest	
			Norm	Decr
Occup insecticide exposure				
in agriculture	28	20	17	3
in horticulture	9	8	7	1
in airplane spraying	20	15	14	1
in manufacturing	12	11	11	
Accidental insecticide exposure	4	4	4	
Anticholinesterase treatment of				
myasthenia gravis	5	3	3	
glaucome	7	1	1	
Hospital patients with				
cirrhosis of the liver	12	10	2	8
moderate exp of CCl_4	2	2	2	
severe lead poisoning	[illegible]	2	1	1
nephrosis	3	3	3	
renal insufficiency	10	5	3	2
tetanus (treatment with suxamenton)	1	1	1	
Healthy adults without insecticide exposure	20	15	12	3
Total	135	100	81	19

with various clinical conditions, and from healthy adults not exposed to cholinesterase inhibitors (Table I) At each sampling venous blood and capillary blood were taken at the same time The venous blood was put directly into tubes and centrifuged after cooling 0 05 ml capillary blood from the finger tip in a heparinized pipette was applied to filter paper and air-dried four such samples were taken If these were not analysed on the same day the filter papers were kept in a refrigerator

Acholest Test

Determination with indicator strip was carried out according to the instructions supplied The analysis was done at room temperature No temperature correction (16) was made

Principle

Serum cholinesterase catalyses the following reaction acetylcholine + H O → choline + acetic acid The test strip is impregnated with a cholinester and an indicator (acid = yellow neutral = blue) On the addition of serum the colour is blue The acetic acid that originates on acetylcholine decomposition reduces the pH which results in a change of colour from blue green through green yellow green to yellow The time interval between the serum contact with the test strip and the agreement of the yellow colour with the accompanying control strip is the measure of the cholinesterase activity

Technique

Two drops of serum each of 0 05 ml from the centrifuged venous blood are placed on a well cleaned object glass With a small forceps half an Acholest strip is placed in one drop and the control strip in the other A stopwatch is started when the Acholest strip is placed in the serum and stopped when the colour on this strip coincides with the colour on the control strip The measured time is the result of the determination The normal BuChE activity is 6–18 min 19–35 min is decreased activity and more than 35 min strongly decreased" activity

Warburg Technique

Whole blood is collected from a punctured finger with a heparinized glass pipette The first drop of the finger tip blood is wiped off with a piece of dry dressing. Exactly 0 05 ml is applied to a filter paper (Munktell no 3) Four blood samples are taken for each analysis The filter papers are air-dried at room temperature Blood samples on paper are kept in a refrigerator (4 C) until the analysis The Warburg manometric technique (1 2, 3) is used to determine the esterase activity in plasma and erythrocytes The enzyme concentration (esterase activity) is expressed in U ml where U is the amount of enzyme that will catalyze the hydrolysis of 1 μmole of substrate per minute The normal BuChE activity values for males are $2\ 20 \pm 0\ 40$ and for females $2\ 03 \pm 0.54$ U ml The normal AChE activity values are $0\ 95 \pm 0\ 16$ and $0\ 89 \pm 0\ 12$ U ml respectively (3)

RESULTS

Fig 1 shows the correlation between BuChE activity determined partly by the Acholest and partly by the Warburg method in 134 samples from 100 persons There is a good correlation between the two methods The spread around the mean value 1 and 2 σ (70 % and 95 %, respectively) for normal Acholest values is $\pm 0\ 36$ and $\pm 0\ 72$ U ml^{-1}

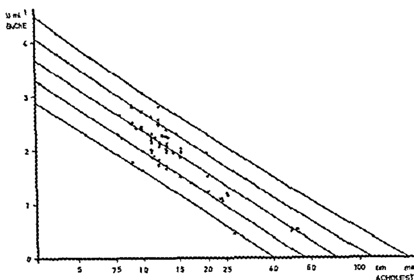


Fig 1 Comparison of the results obtained in 134 blood samples tested for butyrocholinesterase activity (*BuChE*) by the Acholest test and by the Warburg method

respectively In four samples Acholest showed reduced activity (18–21 min) whereas the Warburg method would place these values in the normal range Thirteen samples which lay within normal Acholest values had decreased activity according to the Warburg method the lowest being 1 32 U ml⁻¹

One of the 135 samples as recorded in Table I is not included in Fig 1 because it showed strongly decreased activity The colour change in the Acholest strip then occurs so slowly that it is difficult to decide when the colour begins to agree with that of the control strip Table II shows the results from the person poisoned by an organophosphorus insecticide from whom the omitted sample was taken

Fig 2 shows the correlation between the BuChE activity determined by Acholest and AChE activity in erythrocytes determined by the Warburg method As expected no reliable correlation exists

To investigate whether Acholest can be used for determination of BuChE activity in non fresh serum a daily determination was carried out on the same serum some of which was kept at room temperature and some in a refrigerator (4 C) Over a period of 10–17 days five samples were investigated (Fig 3) Changes of significance in the Acholest results appeared in only one sample (no 1 in Fig 3) In the part of this sample that was kept in the refrigerator the results varied by a maximum of 8 min In the part kept at room temperature considerable variation occurred with Acholest 30 min on the second day and 13 min on the ninth and eleventh days This sample kept at room temperature was the only one that showed a tendency to falling cholinesterase activity during this period In serum from the other four and from those kept up to one week no significant change was found in the Acholest results during the storage period

COMMENTS

The investigation has proved the existence of a satisfactory agreement between Acholest and the Warburg manometric method in the determination of BuChE activity Similar results were obtained earlier between Acholest and the mano

Table II *Cholinesterase activity in blood from a 17 year-old male exposed to organophosphorus insecticide periodically during June and July 3½ h on 3 Aug and 2 h on 4 Aug First symptoms observed on 4 Aug Recovered on 20 Aug*

Warburg method values expressed in U ml⁻¹ i e μmoles of substrate hydrolyzed per min per mole of whole blood

Date		Acholest time in min	Warburg method U ml⁻¹ Plasma	R B C
Aug	5	158	0 05	0
	7	53	0 51	0 06
	11	9	0 90	0 15
	20	19	1 49	0 4
Sept.	9	13	07	0 30

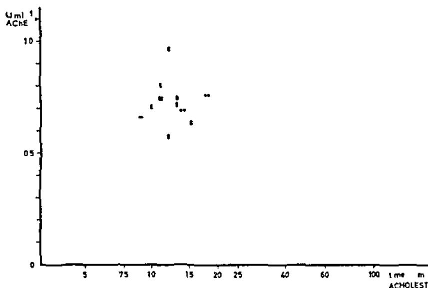


Fig 2 Comparison of the results obtained in 134 blood samples tested for butyrocholinesterase activity (*BuChE*) by the Acholest test and for acetylcholinesterase activity (*AChE*) by the Warburg method

metric method of Ammon (10 17) and between Acholest and the electrometric method of Michel (8 16 20) Although the physiological function of butyrocholinesterase in plasma is not known the determination of its activity is of value for forming an idea of the absorption of cholinesterase inhibitor In connection with the control of workers exposed to insecticides and to nerve gases of the type cholinesterase inhibitor this test can often

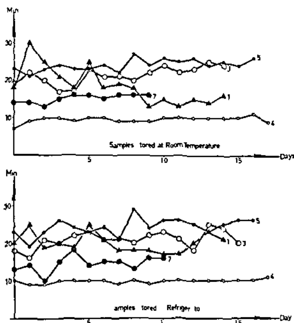


Fig 3 Results of daily Acholest tests on five blood samples stored for a period of 10–17 days at room temperature and in a refrigerator

be of greater value than merely to show the degree of the poisoning by determination of the acetylcholinesterase activity Acholest in this connection is a simple screening test with a satisfactory reliability Acholest provides the important information as to whether the serum cholinesterase activity is normal or decreased and approximately how much it is decreased At normal and slightly reduced cholinesterase activity no difficulties exist in reading when the yellow colour on the Acholest strip agrees with the colour on the control strip At greater reduction it may be difficult to determine when the two strips begin to show the same colour which means that the Acholest value is less precise This however is of less importance Acholest should be used for practical purposes and not for providing exact values in connection with for instance treatment for poisoning or scientific investigations On these occasions there are more exact manometric electrometric and colorimetric methods (7 11) which because of their complicated apparatus and time demanding procedures are unsuitable for screening tests

Acholest does not require laboratory facilities Persons exposed to cholinesterase inhibitors can be controlled by e g an industrial nurse or district nurse If normal values are found the analysis is ready within 30 min It is a big advantage that a response can be obtained so quickly because the work and the exposure should be discontinued if the cholinesterase activity is low At Acholest values of more than 30–40 min the risk for the

poisoned person is so great that continued observation should be carried out in a special clinic

If the analysis cannot be mad at once for instance when samples are taken from a large group Acholest gives unchanged values up to at least on week It is an advantage—although not necessary—that serum be kept in a refrigerator during this period

Approximately 10% of those in our material who were exposed occupationally had reduced BuChE activity Our investigation was not an epidemiological study but the persons investigated were from the same area as those investigated by Tejning in 1959–1960 (19) He found reduced BuChE activity in approximately 50 % of the spraymen Comparison of the two investigations indicates that an improvement had occurred the risk to health had been reduced During the 1960s an increasing amount of information and training has been provided for workers who handle the toxic pesticides as from 1964 permission from the authorities is necessary before these substances can be used However although the risk of poisoning has decreased quantitatively qualitatively it is still too great because of negligence accidents disregard of occupational hygienic measures and unconscious exposure resorption

Of the 15 healthy persons who were included in the normal material (Table I) 12 had normal values with Acholest between 9 and 18 min (2 56–1 58 U ml^{-1} mean 2 14 U ml^{-1}) Repeated tests from the other three were 19 5 min (1 64 U ml^{-1}) between 20 and 32 min (1 33–1 11 U ml^{-1}) and between 22 and 28 min (1 26–0 9 U ml^{-1}) Liver function tests on all three were normal

Physiological hypocholinesterasaemia may occur during pregnancy (7) Familiar hypocholinesterasaemia (12) familiar dyscholinesterasaemia (7 13) and atypical serum cholinesterase (4) have been described

Plasma cholinesterase is formed in the liver and moves electrophoretically with α globulin (95%) and with albumin (5%) The production in the liver of plasma cholinesterase and albumin occurs more or less in parallel a correlation exists between the plasma albumin level and BuChE activity in several diseased conditions (5) Determination of BuChE activity in various hepatic disorders has been reported by several authors (15 17) and has been judged to be a valuable liver function test especially in chronic hepatic disorders such as cirrhosis and chronic hepatitis Of our ten patients with liver cirrhosis eight showed decreased BuChE activity the lowest being 0 4 U ml^{-1} (Acholest 98 min)

Acholest under 5 min is understood to mean increased BuChE activity Hypercholinesterasaemia may occur in adiposis nephrosis and exudative enteropathy (5) In the two latter conditions a compensatory raised albumin synthesis occurs in the liver and parallel with this an increased production of cholinesterase In our material nobody had hypercholinesterasaemia Of those investigated 15 had Acholest values less than 10 min and three less than 8 min there were none less than 6 min Of these 15 three were patients with the nephrotic syndrome Acholest was 6 min (3 15) 8 min (2 85) and 8 min (2 07 U ml^{-1}) AChE activity was normal or low 0 78 0 75 and 0 57 U ml^{-1} respectively

The BuChE activity becomes reduced in connection with dialysis whereas AChE activity remains unchanged (9) Of our five patients investigated for chronic renal insufficiency three had normal and two slightly increased BuChE activity The activity was reduced in connection with dialysis in four patients The reduction was not significant The AChE activity was low in all five with a mean value of 0 37 (0 23–0 58) U ml^{-1} but rose significantly in connection with dialysis to 0 60 (0 44–0 82) U ml^{-1}

CONCLUSION

Those who are exposed to cholinesterase inhibitors in their work are subjected to a serious health risk Because the cholinesterase activity in the body tissue can be reduced as much as 75 before the first subjective symptoms reveal themselves the risk zone may be very small i e the period from the appearance of the first symptom to the point when a life threatening condition exists It is therefore desirable from the occupational hygienic aspect that a regular control of this occupational group be carried out in order to prevent poisoning Because there now exist simple and rapid semi quantitative methods for determining the cholinesterase activity in serum such a control ought to take place

There are hereditary sickness conditions associated with hypocholinesterasaemia therefore tests for determining the cholinesterase activity

should be made before workers enter employment where there is exposure to cholinesterase inhibitors For those who spray pesticides this test can be taken in conjunction with the competence course On seasonal exposure e g in agriculture tests can probably also be taken before the beginning of the spraying season Tests should be taken every week as long as exposure continues At higher exposure tests are advisable two or three times a week In the event of occasional strong exposure for instance in connection with spill on skin and clothing or if suspected poisoning symptoms are present tests should be taken on the same day

Sampling and analysis can be made by a nurse without access to a laboratory If the worker shows reduced cholinesterase activity (Acholest 20–35 min) he can be transferred to non-exposed work When the cholinesterase activity returns to normal he can resume his earlier work At strongly decreased activity (Acholest more than 35 min) a clinic of occupational or internal medicine should take over the responsibility for further measures

Besides the control of workers who are exposed to insecticides and nerve gas s Acholest and similar simple semiquantitative methods can be of value as control tests in the use of muscle relaxants of succinylcholine type in anaesthesia cholinesterase inhibitor in myasthenia gravis glaucoma and cancer also as diagnostic test in hepatic disorders

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REFERENCES

1 Augustinsson K B Acta physiol scand 35 40 1955
2 Augustinsson, K B & Heimbürger G Acta physiol scand 30 45 1953
3 Augustinsson K B & Holmstedt B Scand J clin Lab Invest. 17 573 1965
4 Brody I A Resnick J S & Engel W K Arch Neurol 13 126 1965
5 Faber M Acta med scand 114 59 1943
6 Fišerova Bergerova V XVth International Congress on Occupational Health Vol II 1 p 485 Verlag der Wiener Medizinischen Akademie Wien 1966
7 Goedde H W, Doenicke A & Altland K. Pseudocholinesterasen Springer Berlin 1967
8 Holmes J H & Jankowsky L Arch environm Hlth 13 564 1966
9 Holmes J H Nakamoto S & Sawyer K. C Trans. Amer Soc artif intern organs 4 16 1958
10 Jabsa, Z. Schönfelder M & Brener H Klin. Wschr 39 966 1961
11 Jensen Holm J Kolinesterasers aktivitet og biologiske funktion Borgens Forlag, Copenhagen 1966
12 Klaus D & Renschler H Klin Wschr 38 439 1960
13 Lehmann H Cook J & Ryan E Proc roy Soc. Med 50 147 1957
14 McGavin D D M Lancet 2 27? 1965
15 Pietschmann H Wien Z inn Med 41 409 1960
16 Richterich R Schweiz med Wschr 9? 263 1962.
17 Sailer S & Braunsteiner H Klin Wschr 37 986 1959
18 Schubert F & Kenter J R Anesth Analg Curr Res 41 727 1962
19 Tejning S & Övrum, P Personal communication.
20 Wang R. I & Henschel E O Anesth Analg Curr Res 46 281 1967

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CIRCULATORY STUDIES DURING PHYSICAL EXERCISE IN MENTALLY DISORDERED PATIENTS

I *Effects of Large Doses of Chlorpromazine*

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Abstract 1 Nine patients who were not given chlorpromazine (mean age 29 years) and eight patients (mean age 30 years) who received large doses of chlorpromazine (1500–3600 mg/day) were studied with determination of oxygen uptake ventilation, cardiac output, arterial blood pressure and arterial blood lactate pyruvate and noradrenaline concentrations at rest supine and when cycling in the sitting position on a bicycle ergometer with successively increasing work loads. The heart volume was measured roentgenologically with the patient prone 2 Four patients were studied before and 1.5–5 months after the beginning of chlorpromazine treatment They maintained their physical performance during that period. 3 All patients had a low physical working capacity on the bicycle ergometer and most of them had a reduced stroke volume in relation to the heart volume 4 The patients treated with chlorpromazine tended to show further reduction in stroke volume and lower cardiac output during exercise than those who did not receive such treatment. 5 The arterial blood pressure at rest was largely the same in the two groups of patients, but was reduced during exercise in those treated with chlorpromazine Chlorpromazine did not cause a significant change in peripheral vascular resistance 6 A high blood noradrenaline level was recorded during exercise after treatment with chlorpromazine 7 A small stroke volume especially in patients treated with chlorpromazine and the fall in arterial blood pressure in this group are factors that limit physical performance The importance is stressed of exercise tests in clinical routine in the activation of patients receiving phenothiazines, at least for those receiving large doses

The physical working capacity of mental hospital patients with chronic disorders is often low (5) Such patients may sometimes be treated with large doses of phenothiazines Both the physical inactivity and the drug may substantially impair the adaptation of the circulation to physical exercise and thereby retard rehabilitation It was therefore thought worthwhile to make a thorough physiological study of a group of patients with chronic mental diseases including some patients receiving large doses of chlorpromazine

Long term treatment with chlorpromazine results in reduction of the mean arterial blood pressure in upright posture (18) but no studies have reported on the effect of such treatment on the arterial pressure during exercise Certain unexplained cases of sudden death (9 13) among patients receiving phenothiazines may have been due partly to maladaptation of the circulation Carlsson et al (6) reported an increased noradrenaline concentration at rest in the blood plasma and urine in patients receiving large doses of chlorpromazine The difference in noradrenaline level between such patient and patients not receiving the drug was still more striking during physical exercise

This investigation which is a continuation of the study just mentioned (6) is concerned with the effects of physical inactivity and of large doses of chlorpromazine on circulatory adaptation to physical exercise Patients who had been receiving large doses on clinical grounds were selected for the study Some of the patients took part in a physical training programme reported in the following article in this journal (7)

MATERIAL

The material is summarized in Table I Group I (9 males) mean age 9 years (range 19–42) consisted of patients who had not received chlorpromazine Group II (8 males) mean age 30 years (range 23–50) consisted

Table I *Anthropometrical and clinical data*

Pat no	Age (y)	Weight (kg)	Height (cm)	Diagnosis	Duration of disease (y)	Medicine: Chlorpromazine Dose (mg/day)	Chlorpromazine Duration (mo)	Additional drugs daily dose	Duration
Group I									
1	19	57	170	Schizo-phrenia	3	0	—		
2	37	64	167	Schizo phrenia	20	0	—		
3	42	77	167	Schizo phrenia	20	0	—	Phenytoin (100 mg × 3) pheno-barbitone (100 mg × 3)	2 mo
4	31	57	176	Schizo phrenia	6	0	—	Amylobarbitone 100 mg	Tempo-rarily
5	8	63	176	Schizo-phrenia	6	0	—		
13	19	6_	183	Schizo-phrenia	1	0	—		
14	33	9_	167	Mania	15	0	—		
15	_0	60	174	Schizo-phrenia	1	0	—		
18	30	80	176	Anxiety neurosis	3	0	—		
Group II									
5	28	63	176	Schizo phrenia	6	2000	1 5		
6	31	108	185	Schizo phrenia	11	3000	4		
7	43	87	171	Schizo-phrenia	9	3600	> 1?		
8	23	101	181	Schizo phrenia	3	1800	1		
9	?6	(8	179	Schizo phrenia	6	_500	4		
10	23	0	175	Schizo-phrenia	2	1500	> 12	Benztropine (? mg × 1) *n*-ethyl norphenyl ephrine hydrochloride (Effontil ®) (5 mg × 3)	2 mo and 1 mo resp
11	27	99	181	Schizo phrenia	5	?400	> 12	Benztropine (2 mg × 1) *n*-ethyl norphenyl ephrine hydrochloride (Effontil ®) (20 mg × 3)	_ mo
12	50	69	174	Schizo phrenia	8	_100	> 12	Benztropine (2 mg × 1) amphetamine (5 mg × ?)	2 mo

of those who had received large doses of chlorpromazine for more than one month Most patients had been ill for several years some of them for even more than a decade during which they had been physically rather inactive The patients were informed about the experimental nature of the study and all agreed to cooperate

Four patients (nos 1 2 4 and 5) were studied both before and 1 5–5 months after the beginning of treatment with chlorpromazine with final doses of 300 300 900 and 2000 mg/day respectively Patients 1 2 and 4 had before insertion of the drug taken part in the physical training programme for 1 5–3 months (ref 7) They continued their physical training after application of chlorpromazine and kept their physical activity fairly constant The total number of patients receiving large or rather large doses of phenothiazines in the hospital is considerable But the frequency of new inpatients requiring such large doses of phenothiazines is very low It was considered justifiable however to include this small group in our report

METHODS

The patients arrived at the laboratory in the morning The heart volume was determined roentgenologically with the patient prone (19) Polyethylene catheters were inserted percutaneously (?) into a brachial artery and a

peripheral arm vein The tip of the venous catheter was placed proximally to the axilla The plasma volume was then determined with ^{131}I albumin (RISA) The total blood volume was calculated from hematocrit values obtained for peripheral arterial blood corrected for trapped plasma and for body hematocrit

The cardiac output was determined with the indicator dilution technique using ^{131}I Hippuran (o iodohippuric acid sodium salt, obtained from Radiochemical Centre Amersham, England 20–40 μC in a volume of 1 0 ml for each injection) as indicator and fraction collector for the arterial samples The technique has been discussed by Bjure (4) who found good agreement between the cardiac output determined with the Hippuran method and with the direct Fick method

Resting electrocardiograms comprising the standard leads I II III the augmented unipolar extremity leads aVR aVL aVF and the precordial leads CR CR_3 CR_4 CR_5 CR_7 were recorded on a direct writing mingograph (Elema Sweden) During exercise the indifferent electrode was placed on the forehead (CH leads) and CH_3 CH CH and CH were recorded The heart rate was counted from the electrocardiogram using not less than 25 beats

The intraarterial blood pressure was recorded with an inductance manometer (Elema, Stockholm) and the mean pressure was obtained by electrical integration. When the patient was supine a level, 5 cm below the angle of Ludwig was used as reference and when sitting, the connection of the fourth rib to the sternum

The oxygen uptake was measured by collecting expired air in Douglas bags and analysing it with the micro-Scholander technique

The hemoglobin concentration in arterial blood was determined spectrophotometrically after conversion to cyan methemoglobin, according to Drabkin and Austin (8) The oxygen saturation in arterial blood was determined spectrophotometrically as described by Holmgren and Pernow (16)

Lactic acid was determined on arterial blood according to Strom s (2?) modification of the Baker and Summerson method In most patients it was also estimated by an enzymatic method (1) Pyruvic acid was determined by an enzymatic spectrophotometric method (10)

The noradrenaline concentration in arterial plasma was estimated spectrophotometrically according to the principles of the trihydroxyindole reaction after the samples had been passed through a column of strong cation exchange resin (17)

The hemodynamic studies were started at the earliest two hours after the patients had had their regular breakfast Measurements were first made with the patient resting in the supine position The patients afterwards sat on the bicycle ergometer (14) and the arterial blood pressure was recorded after about 2–4 min in this position Exercise was then started and was done with successively increasing work loads with 10 min pauses during which the patient rested in a chair The BP and ECG were recorded every second min The cardiac output was determined after exercise for 6 min with each load. The BP was recorded immediately before and after determination of the cardiac output The expired air was collected during determination of the cardiac output After exercise for about 8 min with each work load, or occasionally immediately and 3–4 min after a shorter period of work if the patient found the exercise very strenuous blood was collected for measurement of lactic acid and pyruvic acid

After exercise for 9 to 11 min arterial blood was sampled for determination of the noradrenaline concentration

The highest work load was chosen so that the patient would be exhausted after the exercise period. In patients 5 and 11 the highest work load could only be maintained for a short period and the hemodynamic observation was made already after 3–4 min of exercise Such results were not used in the calculation of mean values, but are nevertheless included in Tables II and III All the patients had performed a regular exercise test with recording of the ECG before the examination proper this was also carefully explained to the patients No complications excepting minor hematomas were observed

RESULTS

Comparison between groups I and II

The results obtained for patients who had not received chlorpromazine (group I) and for those who had (group II) are given in Tables II and III Table IV shows the mean values for pertinent data in the two groups

The *highest work load* which could be maintained for at least 6 min usually 10–11 min was on the average 739 (range 300–900) kpm/min in group I and 563 (range 300–900) kpm/min in group II

It was not possible to obtain samples of expired air from all the patients because some of them did not tolerate the mouthpiece or the noseclip In group I the *oxygen uptake* at rest was 114 % (range 84–150) of the predicted basal metabolism (12) and in group II it was 106 (range 90–130) No systematic difference in oxygen uptake at rest or during exercise was observed between the two groups The values during exercise were of the same order as those found in comparable studies of healthy men of similar ages (e g 10 11)

The *total ventilation at rest and during light* exercise was sometimes higher in group II than in group I

The average *arterial hemoglobin concentration* at rest was largely the same in both groups (I = 14 0 and II = 13 7 g/100 ml) The corresponding values noted for the highest work load were 14 7 and 14 6 g/100 ml The *arterial oxygen*

Table II *Results of physiological tests at rest and during exercise in untrained patients without chlorpromazine*

Pat. no	Work load	Oxygen uptake STPD (l/min)	Ventilation BTPS (l/min)	Cardiac output (l/min)	Heart rate (beats/min)	Stroke volume (ml)	Blood pressure (mm Hg)			Lactate (mM/l)	Pyruvate (mM/l)	Noradrenaline (µg/l plasma)
							Syst	Diast	Mean			
1	Rest Supine	0.33	9.6	8.3	90	92	1[illegible]7	66	91	0.88	0.06	0.8
660 ml	Sitting				130		142	83	105			
4.3 l	300	0.61	12.2	12.2	158	77	155	96	116	2.08	0.14	1.4
14.3 g/100 ml	450	1.03	22.9	13.5	187	72	159	91	112	3.53	0.17	2.3
	750	1.41	33.6	13.3	207	64	156	105	121	9.18	0.25	2.7
2	Rest Supine	0.26	6.2	7.0	83	85	131	67	87	0.69	0.04	0.7
	Sitting				96		134	74	93			
600 ml	300	0.86	19.0	8.8	106	83	149	68	94	0.78	0.04	1.3
4.6 l	600	1.45	31.4	10.03	139	74	155	69	95	1.49	0.05	2.6
13.9 g/100 ml	900	2.06	52.3	12.8	171	72	141	73	100	5.33	0.06	2.9
3	Rest Supine	0.[illegible]6	10.1	5.9	74	80	132	75	105	1.56	0.06	0.7
820 ml	Sitting				71		142	87	113			
3.9 l	300	0.83	19.4	9.0	116	78	167	90	116	4.48	0.[illegible]0	1.3
14.7 g/100 ml	500	1.03	30.0	9.2	146	63	175	96	122	6.09	0.23	2.1
4	Rest Supine	0.18	5.4	5.1	69	74	106	56	74	1.08	0.06	0.5
620 ml	Sitting				68		124	69	92			
5.0 l	300	0.83	22.3	10.4	108	96	161	78	108	3.53	0.17	1.3
10.3 g/100 ml	600	1.59	64.9	—	164	—	197	96	137	8.75	0.24	2.6
5	Rest Supine	0.33	10.0	13.3	120	111	144	77	96	0.96	0.06	0.3
730 ml	Sitting				109		141	83	100			
5.5 l	300	0.95	26.1	14.0	144	97	171	97	129	2.38	0.11	0.6
14.5 g/100 ml	600	1.54	45.5	14.9	152	98	165	77	109	4.68	0.15	—
	900	[illegible].19	68.9	17.5	178	98	157	81	118	7.24	0.35	4.0
13	Rest Supine	0.22	5.2	4.6	6[illegible]	74	120	61	81	(0.95)	0.08	
610 ml	Sitting				61		131	74	99			
4.2 l	300	0.89	17.9	10.1	90	114	150	79	109	—	—	
14.0 g/100 ml	600	1.42	30.6	13.5	152	89	179	90	114	(4.33)	0.15	
14	Rest Supine	0.28	7.7	7.3		94	131	70	97	1.44	0.10	
800 ml	Sitting				81		155	90	109			
—	300	0.90	21.3	—	116	—	169	86	113	2.01	0.13	
15.1 g/100 ml	(600)	1.01	26.1	—	124	—	173	81	99	2.04	0.14	
15	Rest Supine	0.[illegible]8	6.9	7.3	77	95	166	91	116	(0.97)	0.07	
—	Sitting				64		156	91	115			
4.1 l	300	1.06	23.4	14.1	113	125	186	104	132	(1.99)	0.13	
15.1 g/100 ml	600	1.49	41.2	18.3	143	128	219	105	143	(4.49)	0.23	
	900			21.1	175	121	22	105	149	(0.64)	0.24	
18	Rest Supine	0.28	7.3	7.9	84	94	125	65	93	0.73	0.07	
920 ml	Sitting				100		165	90	109			
—	300	0.99	[illegible]4.8	10.3	117	88	187	85	119	0.97	0.07	
14.5 g/100 ml	600	1.50	34.0	12.3	141	87	205	86	11[illegible]	1.67	0.12	
	900	1.98	48.4	15.8	172	92	213	80	113	3.38	0.13	

Left column gives subject's number, heart volume, blood volume and hemoglobin concentration at rest

Table III *Results of physiological tests at rest and during exercise in untrained patients receiving large doses of chlorpromazine*

Pat. no	Work load	Oxygen uptake STPD (l/min)	Ventilation BTPS (l/min)	Cardiac output (l/min)	Heart rate (beats/min)	Stroke volume (ml)	Blood pressure (mm Hg) Syst.	Diast	Mean	Lactate (mM/l)	Pyruvate (mM/l)	Noradrenaline (μg/l plasma)
5	Rest Supine	0 30	9 4	6 8	92	74	115	65	84	(0 38)	0 10	1 1
—	Sitting				121		115	65	84			
—	300	0 88	23 1	8 9	130	68	136	67	97	(1 30)	0 08	1 1
13 1 g/100 ml	600	1 55	41 1	11 1	160	70	157	74	107	(4 33)	0 14	5 5
	900	2 13	66 7	14 5	175	83	162	78	120	(6 38)	0 21	7 0
6	Rest Supine	—	—	6 2	81	76	117	75	95	0 71	0 04	
850 ml	Sitting				81		133	99	109			
6 6 l	300	—	—	10 0	120	83	132	80	99	2 03	0 07	
12 5 g/100 ml	600	—	—	12 2	131	93	140	75	99	3 77	0 08	
7	Rest Supine	—	—	7 1	112	83	130	80	98	0 70	0 07	2 0
740 ml	Sitting				114		144	92	114			
4 8 l	300	—	—	10 2	137	74	166	87	115	1 95	0 14	5 4
13 2 g/100 ml	600	—	—	10 5	150	70	177	88	118	5 64	0 16	15 0
8	Rest Supine	0 28	7 8	7 7	100	77	131	78	98	0 95	0 06	0 8
620 ml	Sitting				109		131	80	102			
—	300	0 91	24 9	10 3	132	78	165	79	101	1 86	0 11	3 9
14 6 g/100 ml	600	1 58	51 0	14 5	150	97	143	63	88	3 77	0 14	5 9
9	Rest Supine	0 25	8 7	10 9	120	91	134	71	92	0 78	0 05	0 9
520 ml	Sitting				111		158	91	108			
4 8 l	300	0 89	27 3	10 0	136	74	150	79	104	. 11	0 11	4 1
13 4 g/100 ml	600	1 47	48 4	11 4	152	75	135	60	86	4 33	0 09	4 5
10	Rest Supine	0 24	8 4	6 1	100	61	128	74	94	0 58	0 05	0 9
500 ml	Sitting				105		149	94	109			
3 3 l												
14 2 g/100 ml	300	0 92	29 7	10 0	144	70	176	85	112	2 89	0 14	1 5
11	Rest Supine	0 29	7 7	8 3	98	85	137	85	106	2 10	—	1 5
750 ml	Sitting				122		145	96	127			
7 6 l	300	—	—	10 6	137	77	135	83	100	1 69	—	4 7
14 4 g/100 ml	600	—	—	11 2	158	71	159	76	101	3 50	—	2 7
12	Rest Supine	0 25	9 8	5 3	87	61	145	85	109	1 14	0 09	1 0
860 ml	Sitting				90		139	87	108			
4 8 l												
13 9 g/100 ml	300	—	—	6 5	106	62	119	63	81	3 16	0 15	5 2

Left column gives subject's number, heart volume, blood volume and hemoglobin concentration at rest

saturation was normal both at rest and during exercise. The mean resting values for groups I and II were 97 8 and 97 7%. The values for the highest work load were 97 6 and 98 2, respectively.

Fig 1 shows the *cardiac output* in relation to the oxygen uptake (or work load). The cardiac output varied widely in three subjects not treated with chlorpromazine (nos 1, 5 and 15); it was large during exercise, whereas in some patients in group II (specially no 12) it was relatively small. The mean values in group I were of the

Table IV *Mean values for the two groups I without II with chlorpromazine*

		Rest supine		Exercise—Sitting (300 kpm/min)		(600 kpm/min)		(900 kpm/min)	
		I	II	I	II	I	II	I	II
Oxygen uptake	*n*	9	6	9	4	6	3	3	—
STPD (l/min)	Mean	0 27	0 22	0 88	0 90	1 50	1 53	2 06	—
Ventilation	*n*	9	6	9	4	6	3	3	—
BTPS (l/min)	Mean	7 6	8 6	20 7	26 3	41 3	46 8	56 5	—
$\dot{V}_E/\dot{V}_O$	*n*	9	6	9	4	6	3	3	—
	Mean	28 5	32 5	23 4	29 2	27 3	30 6	27 1	—
Cardiac output	*n*	9	8	8	8	5	5	4	—
(l/min)	Mean	7 4	7 3	11 1	9 6	13 9	11 9	16 8	—
Heart rate	*n*	9	8	9	8	6	6	4	—
(beats/min)	Mean	8[illegible]	99	119	130	148	150	174	—
Stroke volume	*n*	9	8	8	8	5	5	4	—
(ml)	Mean	89	76	95	73	95	81	96	—
Arterio venous oxygen	*n*	9	5	8	3	5	[illegible]	3	—
difference (ml/l)	Mean	38	36	81	90	108	119	137	—
Brachial artery pressure	*n*	9	8	9	8	6	6	4	—
(mm/Hg) systolic	Mean	131	130	166	147	187	15[illegible]	183	—
Brachial artery pressure	*n*	9	8	9	8	6	6	4	—
(mm/Hg) diastolic	Mean	70	76	87	78	87	73	85	—
Brachial artery pressure	*n*	9	8	9	8	6	6	4	—
(mm Hg)	Mean	93	97	115	101	118	100	120	—
TPVR $\left(\frac{\text{mm Hg}}{\text{l/min}}\right)$	*n*	9	8	8	8	5	5	4	—
	Mean	13 5	13 8	10 5	10 7	8 5	8 5	7 2	—
Lactate (mM/l)	*n*	7	7	7	7	4	5	3	—
	Mean	1 1	1 0	[illegible]3	2 2	4 2	4 2	5 3	—
Pyruvate (mM/l)	*n*	9	7	8	7	6	5	4	—
	Mean	0 07	0 07	0 12	0 11	0 16	0 12	0 14	—
Noradrenaline	*n*	5	7	5	7	2	4	2	—
(μg/l plasma)	Mean	0 60	1 17	1 18	3 70	[illegible]60	7 73	3 45	—

same order at rest and during exercise at 600 and 900 kpm/min as those found for sedentary men aged 21–39 by Grimby et al (11) where the corresponding values were 6 9 13 6 and 16 7 l/min The mean values during exercise were lower in group II than in group I

The resting *heart rate* (Fig 2) in both the supine and the sitting position tended to be higher in group II (99 and 108 beats/min) than in group I (82 and 87 beats/min) This tendency was also seen in some patients during exercise at 300 kpm/min but not at heavier work loads One patient (no 1 in group I) showed a special pattern with very high heart rate even during light exercise and a low arteriovenous oxygen difference in relation to the heart rate

The *stroke volume* tended to be smaller in group II than in group I (Fig 3) The maximal stroke volume during exercise was related to the heart volume measured roentgenologically in the prone position (Fig 4) In most patients the stroke volume was small in relation to the heart volume and in 11 of 14 patients it was below the regression line observed by Astrand et al (1) for young healthy men It was also noteworthy (Fig 3) that in five of eight patients in group II the largest stroke volume was recorded during exercise with the largest load but never in any of the patients in group I

The average *arterial systolic pressure* when the patient was resting in the supine position was largely the same in both groups In both groups it increased when the patients sat up it was then 144 mm Hg in group I and 148 mm Hg in group II During exercise however it tended to be lower in group II than in group I In four of eight patients in group II it was even lower when they were exercising at 300 kpm/min than when they were sitting at rest on the bicycle This blood pressure pattern was not seen in group I or in a control material of healthy men of the same ages (10) In Fig 5 the systolic

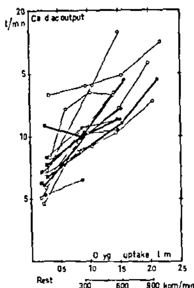


Fig 1 Cardiac output in relation to oxygen uptake (work load) at rest supine and during exercise in sitting position Group I (without chlorpromazine) unfilled rectangles at rest unfilled circles during exercise group II (with chlorpromazine) filled rectangles at rest, filled circles during exercise

blood pressure is related to the heart rate Practically all values in group II fell below the dotted line connecting the mean values found in a control material (10) The *diastolic blood pres*

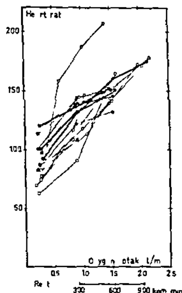


Fig Heart rate in relation to oxygen uptake (work load) at rest supine and during exercise in sitting position. For explanation of symbols see Fig 1

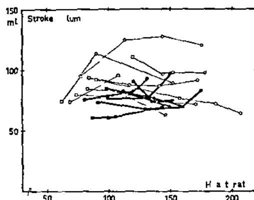


Fig 3 Stroke volume in relation to heart rate at rest supine and during exercise in sitting position For explanation of symbols see Fig 1

sure tended to show a similar difference between the two groups When the patients who were sitting at rest began to exercise at 300 kpm/min the pressure decreased in all of them (except one) in group II but only in three out of nine in group I At rest the *mean arterial pressure* was equal in both groups but during exercise it tended to be lower in group II than in group I In six of eight patients in group II the pressure was lower when they were exercising at 300 kpm/min than when sitting at rest In two of six patients it fell still further when the work load was increased to 600 kpm/min In three patients in group I a similar tendency was noted at the heaviest work load but not at 300 kpm/min

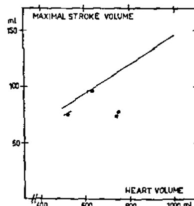


Fig 4 Maximal stroke volume during exercise in relation to heart volume measured in prone position For explanation of symbols see Fig 1 The straight line without symbols gives the relationship in healthy young men reported by Åstrand et al. (1) the broken lines give the standard deviation

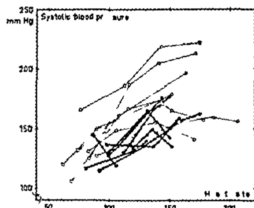


Fig 5 Brachial artery systolic blood pressure in relation to heart rate at rest supine and during sitting exercise For explanation of symbols see Fig 1 The dotted line without symbols connects values in a *group of healthy* men (aged 23–39 years) reported by Grimby (10)

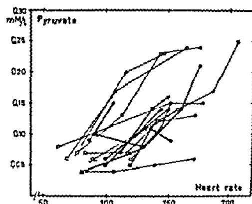


Fig 7 Arterial pyruvate concentration in relation to heart rate at rest supine and during exercise in sitting position. For explanation of symbols see Fig 1 The *dotted line connects mean values in a group of healthy* men (aged 23–39 years) reported by Grimby (10)

In one subject (no 12 in group II) the arterial blood pressure fell markedly during light exercise The stroke volume was small both at rest and during exercise and during exercise the heart rate was relatively low resulting in a small cardiac output during exercise at 300 kpm/min (Fig 1) The lactic acid concentration increased considerably indicating that the oxygen supply to the working muscles was insufficient For this work load the noradrenaline concentration also showed one of the most substantial increases In this patient the blocking effect of chlorpromazine had presumably been unusually strong He was originally selected for physical training but his poor physical performance and the results of the physiological studies induced us not to accept him for this purpose

The *total peripheral vascular resistance* was calculated and the average values for the two groups were practically identical (Table IV) The fall in mean arterial pressure therefore seems to have been due to a relatively small cardiac output

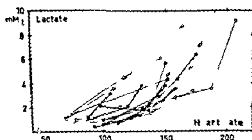


Fig 6 Arterial lactate concentration in relation to heart rate at rest supine and during exercise in sitting position. For explanation of symbols see Fig 1 The dotted line connects mean values in a group of healthy men (aged 23–39 years) reported by Grimby (10)

The *blood lactate concentration* is plotted against the heart rate in Fig 6 In most of the patients in group II the marked increase occurred at a lower heart rate than in a control material (10) or in most subjects in group I Two of the patients in group I (nos 3 and 4) had however high values both at rest and during exercise These two patients together with two others in group I (nos 14 and 15) and one (no 12) in group II had also high values for *pyruvate* whereas in most of the other patients these values were roughly the same as those in Grimby's (10) control material (Fig 7)

The *electrocardiogram* was normal in all patients in group I before during and after exercise In recordings at rest the T waves were flattened in seven of nine patients in group II usually in association with tachycardia No abnormal ECG findings were noted during exercise In three of the patients the flattening of the T waves was less marked four minutes after than before exercise No arhythmia was recorded

Comparison of data before and after commencement of treatment with chlorpromazine

In *four patients comparisons* could be made *before and after treatment with chlorpromazine* had

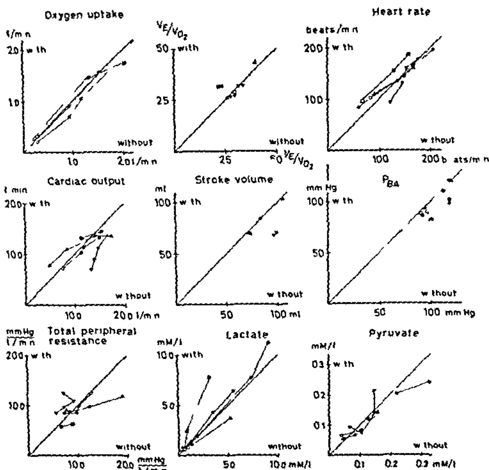


Fig 8 Oxygen uptake ventilatory equivalent, heart rate cardiac output stroke volume brachial artery mean pressure total peripheral resistance lactate and pyruvate concentration without and with chlorpromazine in four patients The straight line is the identity line Unfilled symbols at rest supine filled symbols during exercise in sitting position Patient no 1 (○ ●) no 2 (□ ■) no 4 (△ ▲) no 5 (▽ ▼)

been started (Fig 8) Three of these four patients (nos 1 2 and 4) had taken part in the physical training programme (7) for 1 5–3 months immediately before treatment with the drug They continued their training and maintained their physical performance at a fairly constant level after chlorpromazine treatment had started According to the results in the following paper (7) the physical training may if anything have tended to suppress some of the most important circulatory effects of chlorpromazine namely the high noradrenaline level in blood and the low stroke volume

The *oxygen uptake* did not show any systematic change after treatment with chlorpromazine had been started In three of the four subjects the *ventilatory equivalent* (V_E/V_{O_2}) was higher during exercise after than before administration of chlorpromazine In three of the four subjects the *heart rate* at rest was higher after than before treatment with chlorpromazine had been started During exercise the differences tended to disappear The *cardiac output* did not show any systematic tendency—except lower values at the heaviest work load—after treatment with chlorpromazine had been started During exercise all corresponding values of the *stroke volume* with one exception (no 4 at 300 kpm/min) were lower after than before chlorpromazine had been given This agrees well with the findings when comparing groups I and II All corresponding values for *mean arterial blood pressure* with two exceptions (no 4 at 300 kpm/min and no 5 at 900 kpm/min) were lower after than before

chlorpromazine administration *Total peripheral vascular resistance* however showed fairly wide individual variation but no consistent tendency In three of the four patients when exercising the *lactate concentration* increased more during than before treatment with chlorpromazine The *pyruvate* values did not show any clearcut changes after chlorpromazine therapy had been started

DISCUSSION

The investigation of the circulation at rest and during exercise indicates that large doses of chlorpromazine reduce the arterial blood pressure and the stroke volume which sometimes affects *physical performance* The drug had no demonstrable effect on the total peripheral vascular resistance Hence the fall in mean arterial pressure can probably be ascribed to the small cardiac output This in turn is due to reduction of the stroke volume without a sufficient compensatory increase in the heart rate Small cardiac output and low blood pressure are sometimes seen even during light exercise but at other times occur only during strenuous exercise

The effects of chlorpromazine on the cardiovascular system are complex The drug has not only a direct influence on the heart and blood vessels but also influences them indirectly e g by its action on the central nervous system and autonomic reflexes Hypotension after initial administration may primarily be due to inhibition of centrally mediated pressor reflexes but peripheral α adrenergic blockade may also play a role

The reduction of the stroke volume during light exercise may be due to a decreased vasoconstrictor tone of the capacitance vessels caused by the central and peripheral effects of the drug The normal reaction to exercise is an increase in venomotor tone (3)

The vasoconstrictor tone in the resistance vessels tends at most to be slightly influenced by the drug The primary effect of the drug may perhaps be vasodilatation followed by a compensatorily increased sympathetic activity as an adaptation to the repeated large doses of the drug This may explain the increased noradrenaline outflow but other mechanisms may also be of importance Some of them have been discussed in an earlier paper (6) The increased sympathetic activity however may be insufficient to maintain the stroke volume by normalizing the tone of the capacitance vessels

The effects of adrenergic blocking agents on metabolic processes in man appear to be complex The effects on carbohydrate metabolism in the liver do not readily fall into α or β types of activity but with respect to muscle glycogenolysis and lactacidemic action it seems to be mediated via β receptors (for discussion see ref 20–22)

Noteworthy in this connection are the results of the comparison before and after treatment with the drug which showed that after chlorpromazine treatment the patients—who had higher blood levels of noradrenaline and α receptor blockade to some degree—*in three cases* out of four had higher blood lactate levels during exercise The results suggest that further metabolic studies might be of interest

It may also be questioned whether the high noradrenaline levels in blood do not imply a particular risk for the patients owing to some effect on the β receptors in the heart especially with reference to the sudden death of patients who received large doses of phenothiazines (9 13) In the present study however no remarkable ECG findings were noted except flattening of the T waves

The physical performance must be considered low in both the treated and untreated patients The low stroke volume seems to be one of the factors limiting the physical performance in both groups Both the phenothiazine treatment as discussed above and the long period of physical inactivity can be the causes of the low stroke volume Some patients in group I (nos 1 5 and 15) had a hyperkinetic circulation during exercise with a relatively low arteriovenous oxygen difference even during exercise with the heaviest load This is probably caused by an inadequate regulation of the peripheral blood flow during exercise A similar circulatory dysfunction during exercise is seen in the syndrome vasoregulatory asthenia described by Holmgren et al (15)

The fall in arterial blood pressure during exercise in most patients in group II reduced the tolerance to physical exercise and rendered even the training programme impossible for one of the patients However a fall in blood pressure at rest in the standing position as in the study by Korol et al (18) was not observed in our pa

tients when sitting on a bicycle It should be noted that our patients were well adapted to large dos s The fall in blood pressure during exercise indicated that work tests including re gistration of blood pressure are useful in clinical routine in activation of patients receiving large doses of chlorpromazine Whether similar effects of any significance occur also after treatment with more moderate doses is not yet known A pronounced sudden fall in blood pressure during exertion together with the high noradrenaline level might have been the mechanism involved in the cases of sudden death described by Grei ner et al (9) and Hollister et al (13)

ACKNOWLEDGEMENTS

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REFERENCES

1 Astrand P O Cuddy T E Saltin, B & Stenberg J Cardiac output during submaximal and maximal work J appl Physiol 19 268 1964
2 Bernéus B Carlsten A Holmgren, A. & Seldinger S I Percutaneous catheterization of peripheral ar teri s as a method for blood sampling Scand. J clin Lab Invest 6 217 1954
3 Bevegård, S & Shepherd J Regulation of the cir culation during exercise in man. Physiol Rev 47 178 1967
4 Bjure J Pulmonary diffusing capacity for carbon monoxide in relat on to cardiac output *in man. Scand J clin Lab Invest Suppl 81 1965*
5 Carlsson C Dencker S J, Engstrand I Grimby G & Helander E Phys cal training in the rehabili tation of chronic mentally disabled patients Nord Med 74 788 1965
6 Carlsson C Dencker S J Grimby G & Haggen dal J Noradrenaline in human blood plasma and urine during exercise in patients receiving large doses of chlorpromazine Acta pharmacol (Kbh) 5 97 1967
7 — Circulatory studie during phys cal exerc se in mentally disordered patients II. Effects of physical training in pati nts with and without administration of chlorpromaz ne Acta med scand 184 511 1968
8 Drabkin D L & Austin J H Spectrophotometric studies II Preparat ons from washed blood cells nitric oxide hemoglobin and sulfhemoglobin. J biol Chem 11 51 1935
9 Greiner A C & Nicolson G A Pigment deposi tion in viscera associated with prolonged chlorproma zine therapy Canad med Ass J 91 627 1964
10 Grimby G Exercise in man during pyrogen in duced fever Scand J clin Lab Invest. Suppl 67 1962
11 Grimby G Nilsson N J & Sanne H Repeated serial determination of cardiac output during 30 min exercise J appl Physiol 21 1750 1966
12 Harris J A & Benedict F G A biometric study of basal metabolism in man. Carnegie Institution, Publ no 279 Washington 1919
13 Hollister L E & Kosek J C Sudden death dur ing treatment with phenothiazine derivatives J Amer med Ass 192 1035 1965
14 Holmgren A & Mattsson K H A new ergometer with con tant work load at varying pedalling rate Scand J clin Lab Inve t 6 137 1954
15 Holmgren A Jon son B Levander M Linder holm H Sjostrand T & Strom G Low physical working capacity in suspected heart cases due to inadequate adjustment of peripheral blood flow (vasoregulatory a thenia) Acta med scand 158 413 1957
16 Holmgren A & Pernow B Spectrophotometric measurement of oxygen saturation of blood in the determination of cardiac output. A comparison with the van Slyke method Scand J clin Lab Invest 11 143 1959
17 Haggendal J An improved method for fluorimetric determination of small amounts of adrenaline and noradrenaline in plasma and tissues Acta physiol scand 59 242 1963
18 Korol B Land W J & Brown, M J Effects of chron c chlorpromazine administration on systemic arterial pressure in schizophrenic patients relation ship of body position to blood pressure Clin Pharmacol Ther 6 587 1965
19 Larsson H & Kjellberg S R Roentgenological heart volume determination with special regard to pulse rate and the position of the body Acta radiol (Stockh) 29 159 1948
20 Lundholm L Mohme Lundholm E & Svedmyr N Metabolic effects of catecholamines In Biological basis of medicine chapter 19 Academic Press Lon don 1968 In print
21 Lundholm L Mohme Lundholm F & Vamo N Lactic acid assay with L(+)lactic acid dehydrogenase from rabbit muscle Acta physiol scand 58 243 1963
22 Strom, G The influence of anoxia on lactate util za tion in man after prolonged muscular work Acta physiol scand 17 440 1949

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CIRCULATORY STUDIES DURING PHYSICAL EXERCISE IN MENTALLY DISORDERED PATIENTS

II *Effects of Physical Training in Patients with and without Administration of Chlorpromazine*

Carl Carlsson Sven J Dencker Gunnar Grimby and Jan Haggendal

From Department II Lillhagen Hospital Lillhagen and the Departments of Clinical Physiology and Pharmacology University of Goteborg Goteborg Sweden

Abstract 1 Circulatory studies were performed on four schizophrenics (mean age 27 years) not treated with chlorpromazine and on five (mean age 30 years) treated with large doses of this drug (1800–3600 mg/day) before and after 1 5–4 months of physical training 2 The physical training consisted of strenuous exercise on a bicycle ergometer for three 6-minute periods five times a week 3 After training, all the patients except two could cycle with a larger work load for 6–10 minutes 4 In the dru free patients the heart rate and arterial blood lactate decreased with training This training ef fect was less consistent in the patients treated with chlorpromazine 5 The stroke volume was small in rela tion to the heart volume before training. It tended to become normal in five of eight patients after training. 6 In the patients treated with chlorpromazine the blood noradrenaline level was high during exercise and fell in all the patients except one after training In the dru free patients this level was not as h gh and there was a further decrease with training in all four patients.

In the preceding paper (4) the physical per formance of a group of patients with chronic mental disorders was reported as low Physical inactivity was assumed to be one of the main causes of this weakness Some of the patients were receiving large doses of chlorpromazine which might have contributed to their poor physical performance In an earlier investigation we found that even patients with severe mental disease could take part in a physical training programme with good results (2)

This paper is concerned with the effect of a short period of physical training on the circula tion during exercise In normal men such train ing has been shown to increase the stroke volume (5 6 10) and the arteriovenous oxygen difference (5 10) resulting in an increased maximal cardiac output and aerobic power (maximal oxyg n up take) In patients with coronary disease increased arteriovenous oxygen difference and decreased cardiac work was observed at submaximal work loads after training (11) It was of special inter est to study the effects of training on the pa tients receiving large doses of chlorpromazine According to a previous study (3) training tended to lower the high blood level of noradrenaline in these patients

The present series consists of only a few pa tients This is because as a rule patients of this type cannot manage without drugs for several months In the group given drugs only those pa tients were included who had been treated almost exclusively with one drug (chlorpromazine) in a large dose This also limited the number of pa tients available Despite this selection such pa tients form a rather heterogeneous group Our results should therefore be regarded partly as case reports of patients taking part in a physical training programme

MATERIAL

Four male schizophrenics not treated with chlorproma zine (nos 1 2 4 and 13 in reference 4)—mean age 27 years—and five male patients treated with the drug (1800–3600 mg/day nos 6 7 8 9 and 11 in reference 4)—mean age 30 years—took part in the phys cal train ing programme They were studied before and after 3–4 months of training, except for nos 1 4 and 13 who had to be studied already after 1 5–3 months training, be cause they could no longer manage without chlorproma

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Abstract 1 Circulatory studies were performed on four schizophrenics (mean age 27 years) not treated with chlorpromazine and on five (mean age 30 years) treated with large doses of this drug (1800–3600 mg/day) before and after 1.5–4 months of physical training. 2. The physical training consisted of strenuous exercise on a bicycle ergometer for three 6-minute period five times a week 3 After training, all the patients, except two could cycle with a larger work load for 6–10 minutes 4 In the drug free patients the heart rate and arterial blood lactate decreased with training This training effect was less consistent in the patients treated with chlorpromazine 5 The stroke volume was small in relation to the heart volume before training. It tended to become normal in five of eight patients after training. 6 In the patients treated with chlorpromazine the blood noradrenaline level was high during exercise and fell in all the patients except one after training. In the drug free patients this level was not as high, and there was a further decrease with training in all four patients.

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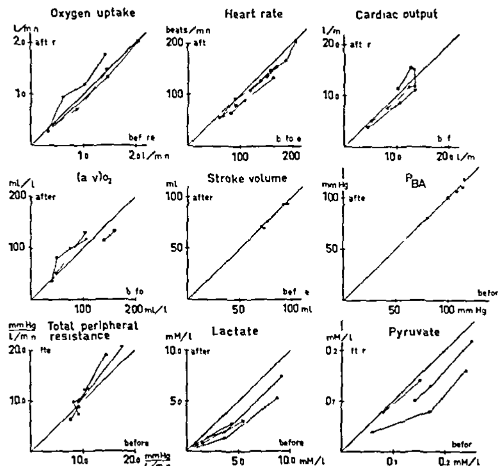


Fig 1 Group I (not treated with chlorpromazine) Oxygen uptake heart rate cardiac output arteriovenous oxygen difference stroke volume brachial artery mean pressure total peripheral resistance arterial lactate and pyruvate concentrations before and after physical training Unfilled symbols at rest supine filled symbols during exercise in sitting position Patient no 1 (○ ●) no 2 (□ ■) no 4 (▽ ▼) no 13 (△ ▲) The identity line is drawn

zine The age height weight of the patients as well as the doses and duration are shown in Table I in the preceding paper (4)

METHODS

The same physiological methods and procedure were used as in the preceding article (4) Oxygen uptake cardiac output (indicator dilution technique) arterial blood pressure arterial blood lactate pyruvate and noradrenaline concentration were determined while the patient was at rest in the supine position and while cycling on an ergometer in the sitting position with stepwise increased work loads up to the highest which the patient could tolerate for 10–11 min

The heart volume was determined roentgenologically in the prone position (9)

The physical training consisted of exercise on a bicycle ergometer five days a week. The patients exercised at the highest or nearly highest work load which they could maintain for six min. On each occasion the patients cycled for three six minute periods at six minute intervals during which they often walked played with a ball etc The heart rate during exercise was regularly recorded and the training load was increased with increasing physical performance

All the patients had performed a preliminary work test some days before the actual physiological studies.

RESULTS

The results obtained in group I (without chlorpromazine) and in group II (with large doses of chlorpromazine) are shown in Figs 1 and 2

In group I the *largest work load* that the patients could maintain for at least 6 min (usually 10–11 min) was on the average 713 kpm/min (range 600–900) before training. After training it increased in three of the four patients and was on the average 900 kpm/min (range 750–

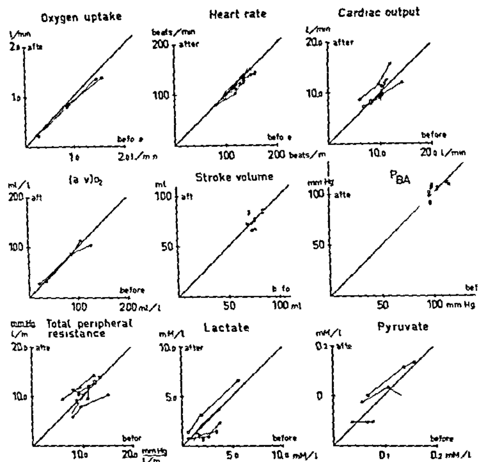


Fig 2 Group II (treated with chlorpromazine) Oxygen uptake heart rate cardiac output, arteriovenous oxygen difference stroke volume brachial artery mean pressure total peripheral resistance arterial lactate and pyruvate concentration before and after physical training Unfilled symbols at rest supine filled symbols during exercise in sitting position Patient no 6 (○ ●) no 7 (□ ■) no 8 (△ ▲) no 9 (▽ ▼) no 11 (▯ ▮) The identity line is drawn.

1050) In group II the corresponding values were 600 kpm/min (for all patients) and 780 kpm/min (range 600–900) After training performance was higher in all patients except one Mechanical efficiency did not seem to change substantially with training as is apparent from the *oxygen uptake* (Figs 1 and 2)

In group I with the exception of patient 13 at 300 kpm/min the *heart rate* was lower in all four patient both at rest and during exercise after training than before training The average difference was at rest 10 beats/min at 300 kpm/min 8 beats/min and at 600 kpm/min 20 beats/min No such tendency was observed at rest in group II where also the average heart rate was considerably higher than in group I (102 compared with 76 beats/min) In three of the five patients the heart rate at 300 and 600 kpm/min was lower after training

With the exception of patient 2 in group I and patient 6 in group II the *cardiac output* was smaller after than before training at the same work load—the results at the heaviest work load were then disregarded There was a tendency (5 of 8 patients) also at rest for cardiac output to be lower after training In three of four patients in group I and in four out of five in group II the highest values measured were higher after than before training

In patients 1 and 13 in group I the *arteriovenous oxygen difference* increased after training. It decreased in patient 2 and was largely unchanged in the other two patients in group II where oxygen uptake was measured

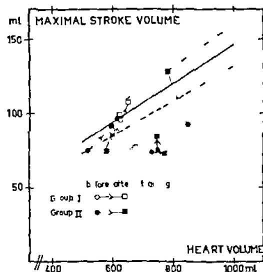


Fig 3 Maximal stroke volume during exercise in relation to heart volume in prone position Data before and after training are connected for each individual in groups I and II The straight line without symbols gives the relationship in healthy young men as reported by Astrand et al (1) The standard deviation is shown with broken lines

In patients 1 and 2 training tended to increase the *stroke volume* In no 1 this increase was seen only at rest In group II this tendency was observed in no 6 and for the heaviest work load also in nos 7 9 and 11 In two of four patients in group I and in three of five patients in group II the maximal stroke volume during exercise was larger than before training (see Fig 3) In the rest of the patients—except no 8—it was unchanged or slightly lower after training In Fig 3 the maximal stroke volume during exercise is also related to the roentgenologically determined heart volume in the prone position The figure shows that the stroke volume tended to become normal in five of eight patients but also that the effect of training varied from patient to patient Training had no demonstrable effect on the *heart volume* and the *plasma and blood volumes*

In some of the patients in group II (nos 8 9 and 11) the *mean arterial pressure* fell during exercise with a lower value at the highest work load than at rest in supine position (Figs 4 and 5) None of the patients in group I showed such a decrease There was no apparent training effect.

The *arterial lactate concentration* was lower after than before training in all patients in group I In group II a similar tendency was observed in patients 6 9 and 11 The *arterial pyruvate concentration* also showed a similar though slighter tendency

The *noradrenaline level* in arterial blood plasma (Figs 6 and 7) fell after training in all patients except in no 8 The fall in pressure was most marked in the other three subjects examined in group II where also the highest levels were found Afte training patient no 8 did not show any decrease in heart rate or in lactate during exercise

DISCUSSION

Training produced a demonstrable effect in both groups of patients but change in adaptation of circulation to exercise was more evident in the patients not treated with chlorpromazine The daily reports on the patients physical performance

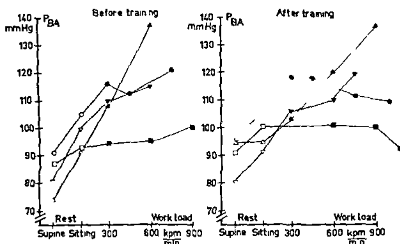


Fig 4 Brachial artery mean pressure ($\bar{P}_{BA}$) in group I at rest supine and sitting and during exercise Left half of the figure shows results before and right half results after traning For explanation of symbols see Fig. 1

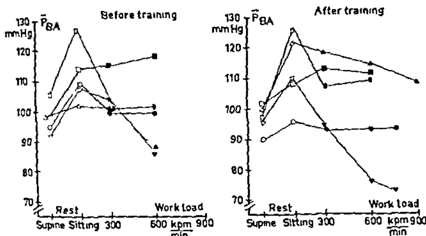


Fig 5 Brachial artery mean pressure ($\bar{P}_{BA}$) in group II at rest supine and sitting and during exercise Left half of the figure shows results before and right half results after training For explanation of symbols see Fig 2

indicated also that in both groups the power of endurance and the maximal tolerable work load increased Part of the increase in the highest work load may well have been due to neuromuscular factors without any substantial change in the circulatory capacity

Our findings agree in general with the results of the pilot study (2) where training was accompanied by a considerable increase in physical performance The present study showed a decrease in heart rate and in lactate and noradrenaline concentrations after exercise A reduced lactate concentration during exercise after training is a common finding (e g 5 10 11) It can be caused by a better relationship between blood flow and metabolism in the working muscle at the same work load but also by a changed removal rate It may indicate increased power of endurance and well trained athletes have even a lower lactate concentration than untrained persons at the same relative work load (the same percentage of the maximal oxygen uptake) (7)

In the present material the decrease in heart rate with training which is also a common finding (e g 5 8 10 11) was not combined in all patients with identical changes in the adaptation of the circulation to exercise Some patients had before training a hyperkinetic circulation with probably a better peripheral regulation of the

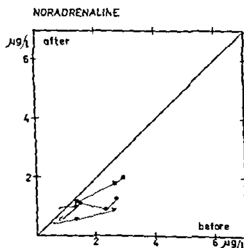


Fig 6 Noradrenaline concentration in blood plasma before and after training in group I. For explanation of symbols see Fig 1 The identity line is drawn.

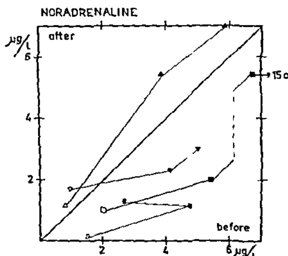


Fig 7 Noradrenaline concentration in blood plasma before and after training in group II. For explanation of symbols see Fig 2 The identity line is drawn.

circulation and a lower cardiac output after training (cf 5 11) whereas others showed an increased stroke volume with training In some recent training studies in young healthy men *more drastic effects have been achieved (5 10)* Even if the training in our study must be regarded as strenuous it was still more strenuous in the investigation reported by Saltin et al (10)

The fall in noradrenaline level during exercise after training is of special interest According to Vendsalu (12) a given work load for a given length of time elicited an approximately similar rise in the blood noradrenaline level In his report no effect due to training was observed In our patients however noradrenaline concentration was reduced during exercise after training both in patients treated with chlorpromazine and in those who had received no such therapy This may be of clinical importance if the very high noradrenaline level in treated patients infers a risk of circulatory disturbances

It is possible that the effect of training on the noradrenaline blood levels is due to the fact that trained muscles contain less lactic acid and other metabolites during work The metabolites may cause dilatation with consequent compensatory increase of adrenergic activity These metabolites may also have an effect on the destruction of the released noradrenaline e g on the re uptake mechanism by the membrane pump On the other hand in the chlorpromazine treated patients the high blood noradrenaline levels before training may have had metabolic effects Thus the arterial blood lactate and noradrenaline decreased after training

The present results and our experience during the investigation show that physical training can be successfully carried out in patients suffering from severe mental disorders who have been inactive for a long time Treatment with large doses of chlorpromazine probably limits the effect of training on the adaptation of the circulation to exercise The suppressive effect of training on the very high noradrenaline level in these patients seems to indicate that it is necessary that even chlorpromazine treated patients get adequate physical exercise For these patients β receptor blocking drugs may possibly be useful for reducing the risk of heart complications in association with training

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REFERENCES

1 Astrand P O., Cuddy T E., Saltin B & Stenberg, J Cardiac output during submaximal and maximal work J appl Physiol 19 268 1964
2 Carlsson, C., Dencker S J Engstrand I Grimby G & Helander E Physical training in the rehabilitation of chronic mentally disabled patients Nord Med 74 788 1965
3 Carlsson, C Dencker S J Grimby G & Haggendal J Noradrenaline in human blood plasma and urine during exercise in patients receiving large doses of chlorpromazine Acta pharmacol (Kbh) 25 97 1967
4 — Circulatory studies during physical exercise in mentally disordered patients I Effects of large doses of chlorpromazine Acta med scand *184 499 1968*
5 Ekblom, B Effect of training on circulatory response to exercise In Physical activity in health and disease Scandinavian University Books Oslo 1966
6 Frick M H Konttinen A & Sarajas H S S Effects o physical training on circulation at rest and during exercise Amer J Cardiol 17 147 1963
7 Grimby G & Saltin, B Physiological analysis of well trained middle aged and old athletes *Acta med scand 179 513 1966*
8 Holmgren A Mossfeldt F Sjostrand T & Strom G Effect of training on work capacity total hemoglobin blood volume heart volume and pulse rate in recumbent and upright position. Acta physiol. *scand 50 72 1960*
9 Larsson H & Kjellberg, S R Roentgenological heart volume determination with special regard to pulse rate and the position of the body Acta radiol (Stockh) 29 159 1948
10 *Saltin B Blomqvist G Mitchell J H Johnsson,* R. C Jr Wildenthal K. & Chapman C B Response to submaximal and maximal exercise after bed rest and training Circulat Res In print.
11 Varnauskas L Bergman H Houk P & Bjorntorp P Haemodynamic effects of physical *training* in coronary patients Lancet 2 8 1966
12 Vendsalu A Studies on adrenaline and noradrenaline in human plasma Acta physiol scand Suppl 173 1960

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BLOOD VOLUME AND EXCHANGEABLE SODIUM IN ESSENTIAL HYPERTENSION

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Abstract 1 No significant difference in total blood volume was demonstrated between patients with untreated essential hypertension and normotensive controls In hypertensive women the mean blood volume was 2317 ml/m² or 63.0 ml/kg as against 2259 ml/m² or 59.2 ml/kg in controls. The corresponding figures for hypertensive men were 2768 ml/m² or 67 1 ml/kg and 2591 ml/m² or 66 0 ml/kg for controls

2. Exchangeable sodium was significantly increased in men with essential hypertension 1736 mEq/m² or 42 4 mEq/kg while 1639 mEq/m² or 39.2 mEq/kg was found in normotensive controls Increased exchangeable sodium was also demonstrated in hypertensive women, 1502 mEq/m² or 41.2 mEq/kg in comparison with normotensive controls whose values were 1445 mEq/m² or 39 9 mEq/kg However because of a difference in height and weight between the hypertensive and the normotensive women the rsults must be taken with reservation.

While a number of studies on changes in blood volume and exchangeable sodium in hypertensive patients on various antipressor regimens have been reported (2 10 11 22 23 25) only a few standardized studies of these parameters in untreated hypertensive patients and normotensive controls have been undertaken and the results presented have often been divergent Some investigators have found a reduced blood volume in essential hypertension (18 19) whereas others have found that it was normal (5 28) Similar disagreement exists in regard to exchangeable sodium where both normal (7 17) and increased (6 20 27) values have been reported Differences in the composition of the control groups could be one of the factors responsible for these contradictory results Blood volume and exchangeable sodium expressed in ml/kg body weight decrease with increasing body weight (9 14 17 24) and adipose tissue in women contains less blood than in men (1 3 14 17) Blood volume does not seem however to vary with age (4 14 26) If blood volume and exchangeable sodium are to be compared in different groups then these groups must be comparable in regard to height weight and sex. Moreover other conditions which might influence these parameters such as renal insufficiency edema congestive heart failure and hematologic diseases must be excluded The purpose of the present study is to compare blood volume and exchangeable sodium in patients with untreated essential hypertension with these values in normotensive controls

MATERIAL AND METHODS

Total blood volume was measured in 22 patients (12 men and 10 women) with untreated essential hypertension and in 20 normotensives (10 men and 10 women) Exchangeable sodium was measured in 15 patients (10 men and 5 women) with essential hypertension and in 19 normotensive controls (1_ men and 7 women) Only hospitalized subjects were investigated and none of them showed evidence of reduced kidney function fluid retention congestive heart failure or hematologic disease All the hypertensive patients had a diastolic blood pressure over 100 mm Hg. Blood pressure and eyeground findings in the hypertensive patients are given in Tables I III VI and VIII All the test subjects had been on a diet containing approximately 6 g of sodium for not less than three days and all the studies were made in the fasting state on patients who had been resting in bed for at least three hours.

Total blood volume was measured as the sum of erythrocyte and plasma volume with a double isotope dilution technique using ^{51}Cr and ^{131}I albumin and ^{24}Na was used in measuring 24-hour exchangeable sodium, as previously described (10 12)

RESULTS

Blood volume

Results in ten hypertensive women (Table I) were compared with those obtained in a group of ten

Table I *Blood volume in hypertensive women*

Pat no	Height (cm)	Weight (kg)	Surface area (m²)	Eye grounds	Blood pressure (mm Hg)	Red cell volume (ml)	Plasma volume (ml)	Total blood volume (ml)	(ml/m²)	(ml/kg)
1	162	50 5	1 52	II	200/110	938	2542	3480	2362	69
2	159	65 2	1 67	II	165/105	1549	2821	4370	2613	67
3	165	66 4	1 73	II	170/115	1727	3097	4824	2786	73
4	160	64 2	1 71	II	180/110	1505	2591	4096	2389	63
5	163	91 0	1 97	II	155/105	1782	2880	4662	2370	50
6	167	54 5	1 61	II	200/115	1473	2315	3786	2358	69
7	15_	59 5	1 56	II	160/100	1361	2156	3517	2258	59
8	153	54 7	1 51	II	170/105	1122	1891	3013	1996	56
9	155	52 5	1 50	II	170/110	1019	_036	3055	2040	58
10	154	53 8	1 51	III	200/105	1087	1916	3003	1993	56
Mean	159 0	61 2	1 63			1356	2425	3781	2317	63 0
±SD	5 3	11 9	0 15			301	426	685	240	7 4
Coefficient of variation									10 3	11 7

normotensive control patients (Table II) Average height weight and surface area are almost identical in the two groups Body surface was calculated using Du Bois formula (8) and blood volume is given as total ml ml/m surface area and ml/kg body weight Blood volume averaged 58 ml/m or 3 8 ml/kg more in the hypertensive women than in the controls This difference however is not significant

Tables III and IV give the results in 12 hypertensive and ten normotensive men Here too average height weight and surface area are almost identical in the two groups Blood volume in the hypertensives averaged 177 ml/m or 1 1 ml/kg mo e than in the controls but the difference is not significant It can be seen from Tables I II III and IV that blood volume in ml/m is a better parameter than blood volume in ml/kg as the coefficient of variation is a slightly lower for ml/m² Table V gives the mean value for blood volumes found in the present study together with those reported by other investigators Individual values in ml/m and ml/kg are illustrated in Fig 1 Here it can be seen that blood volume in men is on the average larger than in women especially if expressed in ml/m

Exchangeable sodium

Results in five hypertensive and seven normotensive women are shown in Tables VI and VII Unfortunatelv it was not possible to obtain two

Table II *Blood volume in normotensive women*

Pat. no	Height (cm)	Weight (kg)	Surface area (m²)	Red cell volume (ml)	Plasma volume (ml)	Total blood volume (ml)	(ml/m²)	(ml/kg)
1	160	62 7	1 65	1306	2560	3866	2339	6?
2	163	63 4	1 68	1376	2939	4315	2564	68
3	161	58 5	1 61	1000	2188	3188	1978	54
4	161	57 3	1 60	1502	2556	4065	2544	71
5	149	51 0	1 44	1040	1831	2871	1997	56
6	161	70 0	1 74	1367	2317	3684	2431	53
7	170	62 5	1 72	1308	_842	4150	2090	65
8	154	58 8	1 56	1305	2282	3587	2293	61
9	160	65 3	1 68	1309	2202	3511	2256	49
10	160	64 4	1 67	1208	2181	3389	2027	53
Mean	159 9	61 2	1 63	1273	2390	3663	2259	59
±SD	5 4	5 2	0 09	154	335	449	221	7 3
Coefficient of variation							9 9	10 3

Table III *Blood volume in hypertensive men*

Pat. no	Height (cm)	Weight (kg)	Surface area (m²)	Eye grounds	Blood pressure (mm Hg)	Red cell volume (ml)	Plasma volume (ml)	Total blood volume (ml)	(ml/m²)	(ml/kg)
1	168	73.8	1.86	II	220/110	1761	2284	4645	[illegible]675	62
2	191	90.5	[illegible].20	I–II	160/110	2240	3516	5756	2621	67
3	180	100.0	2.20	II	180/105	2422	3531	5953	2711	59
4	185	87.5	2.1[illegible]	II	175/110	24[illegible]5	349[illegible]	5917	2797	68
5	169	83.5	1.94	II	160/110	2158	2987	5145	2649	62
6	177	80.6	1.98	II	175/120	2192	3449	5641	[illegible]851	60
7	176	73.5	1.89	II	195/110	2326	3559	5885	3106	80
8	160	60.0	1.62	II	165/110	2096	2890	4986	3074	70
9	170	77.2	1.89	II	175/115	1878	[illegible]638	4516	2393	58
10	160	59.3	1.61	II	215/115	1825	3062	4887	3028	83
11	173	74.8	1.89	II	180/125	1817	3419	5236	2777	68
12	172	67.7	1.80	III	230/120	1591	2968	4559	2533	68
Mean	173.4	77.5	1.92			2061	3[illegible]00	5260	2768	67.1
±S.D.	9.2	11.9	0.19			309	325	551	219	8.1
Coefficient of variation, %									7.9	12.2

Table IV *Blood volume in normotensive men*

Pat. no	Height (cm)	Weight (kg)	Surface area (m²)	Red cell volume (ml)	Plasma volume (ml)	Total blood volume (ml)	(ml/m²)	(ml/kg)
1	176	64.3	1.79	1772	2868	4640	25[illegible]9	73
2	176	72.0	1.88	2113	3415	5528	2943	76
3	177	74.5	1.91	1817	2694	4511	2358	61
4	170	69.1	1.80	1790	2794	4584	2547	68
5	174	71.3	1.85	1936	2862	4798	2587	67
6	171	76.0	1.88	1960	2971	4931	2620	65
7	171	88.3	2.01	2181	4025	6[illegible]06	3091	70
8	164	68.8	1.75	1515	[illegible]340	3855	2208	56
9	19	93.0	2.23	2143	3285	5428	[illegible]434	58
10	179	93.5	2.12	1975	3395	5370	2527	57
Mean	175	77.1	1.92	1920	3065	4985	2591	65.1
±S.D.	7.4	10.6	0.15	207	475	663	259	7.3
Coefficient of variation							9.9	11.2

Table V *Blood volume (mean value) in hypertensive and normotensive patients*

	Blood volume (ml/m²) Hypertensive ♀	♂	Normotensive ♀	♂	(ml/kg) Hypertensive ♀	♂	Normotensive ♀	♂
Present series	317	2768	2[illegible]59	2591	63.0	67.0	59.2	66.0
Rochlin et al 1960	—	—	—	—	61.0	67.5	70.0	76.1
	♀ + ♂		♀ + ♂		♀ + ♂		♀ + ♂	
Reilly et al 1954	[illegible]370		[illegible]490		65.3		65.5	
Walser et al 1956	—		5[illegible]0		58.4		63.1	

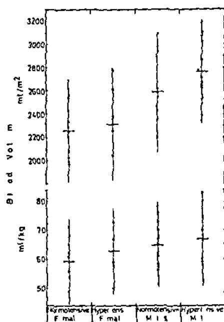


Fig 1 The relation of blood volume to weight and surface area in normotensive and hypertensive men and women The horizontal line represents the mean value for all patients the vertical line gives ±2 S D

fully comparable groups The hypertensive patients weighed on the average 2 7 kg less than the normotensives and were 1 7 cm shorter The relationship between height and weight was however almost identical for the two groups Exchangeable sodium averaged 1502±126 mEq/m for the hypertensives and 1445±78 mEq/m for the controls Expressed in mEq/kg the figures are 41 2±1 8 and 38 9±1 6 Thus the hypertensive women had an exchangeable sodium that was 57 mEq/m greater than that of the controls the difference was not however significant (S E 50 $p > 0\ 2$) The difference in mEq/kg was just on the borderline of significance (S E 1 1 $0\ 05 < p < 0\ 1$) But because of the above mentioned difference in height and weight between the two groups comparisons between them must be taken with reservation

Tables VIII and IX show exchangeable sodium in ten hypertensive and 12 normotensive men The difference in height and weight is so small that comparison is possible Exchangeable sodium was 1736±95 mEq/m in the hypertensive patients and 1639±111 mEq/m² in the controls This difference of 97 mEq/m is significant (S E 47 $p < 0\ 05$) Expressed per kg body weight the figures are 42 4±4 9 and 39 2±2 0 and the difference of 3 2 is also significant (S E 1 5 $p < 0\ 05$) As can be seen in Table VIII the coefficient of variation was lower for exchangeable sodium in mEq/m than per kg in the hypertensive men indicating that exchangeable sodium in mEq/m² is the better parameter The opposite is true in the other groups studied (Tables VI VII and IX) Fig 2 illustrates individual values for exchangeable sodium in mEq/m and per kg in both hypertensive and control patients Most notable here is the fact that men have on the average a larger amount of exchangeable sodium than women

DISCUSSION

In the present study no significant difference in blood volume was observed between hypertensive patients and normal controls Comparison with results obtained by other investigators is difficult, particularly because of different way of selecting

Table VI *Exchangeable sodium in hypertensive women*

Pat no	Height (cm)	Weight (kg)	Surface area (m²)	Eye grounds	Blood pressure (mm Hg)	Exchangeable sodium (mEq)	(mEq/m²)	(mEq/kg)
1	159	65 2	1 67	II	165/105	2856	1707	43 8
2	161	63 5	1 67	II	170/110	2557	1532	40 3
3	154	50 5	1 47	III	170/125	2101	1429	41 6
4	155	52 5	1 50	II	170/100	2185	1459	41 6
5	154	53 8	1 51	III	205/105	2085	1384	38 8
Mean	156 6	57 1	1 56			2357	1502	41 2
±S D	3 2	6 7	0 10			244	126	1 8
Coefficient of variation %							8 3	4 3

Table VII *Exchangeable sodium in normotensive women*

Pat. no	Height (cm)	Weight (kg)	Surface area (m^2)	Exchangeable sodium (mEq)	(mEq/m^2)	(mEq/kg)
1	164	58.5	1.63	2406	1473	41.1
2	160	62.7	1.65	2476	1498	39.9
3	161	58.5	1.61	2256	1399	38.6
4	149	51.0	1.64	2061	1433	40.0
5	160	65.3	1.68	2335	1388	39.9
6	154	58.5	1.56	2343	1498	35.7
7	160	64.4	1.67	2381	1425	37.0
Mean	158.3	59.8	1.63	2322	1445	38.9
±S.D.	5.1	4.8	0.04	134	78	1.6
Coefficient of variation %					5.4	4.1

Table VIII *Exchangeable sodium in hypertensive men*

Pat. no	Height (cm)	Weight (kg)	Surface area (m^2)	Eye-grounds	Blood pressure (mm Hg)	Exchangeable sodium (mEq)	(mEq/m^2)	(mEq/kg)
1	191	90.5	2.20	I–II	160/110	3705	1687	40.9
2	180	100.0	2.20	II	180/105	3683	1678	36.8
3	185	87.5	2.12	II	175/110	3705	1752	42.3
4	169	83.5	1.94	II	160/110	3253	1675	39.0
5	170	77.2	1.89	II	175/115	3093	1634	39.9
6	160	59.3	1.61	II	215/115	3101	1921	52.3
7	173	74.8	1.89	II	180/125	3[illegible]10	1703	42.9
8	175	76.0	1.91	III	220/120	3626	1895	47.7
9	173	100.8	2.14	III	195/145	3676	1718	36.5
10	172	67.7	1.80	III	230/120	3059	1700	45.5
Mean	174.8	81.7	1.97			3410	1736	42.4
±S.D.	8.7	13.4	0.19			290	95	4.9
Coefficient of variation							5.4	11.3

Table IX *Exchangeable sodium in normotensive men*

Pat. no	Height (cm)	Weight (kg)	Surface area (m^2)	Exchangeable sodium (mEq)	(mEq/m^2)	(mEq/kg)
1	171	76.0	1.88	3150	1674	41.4
2	171	88.5	[illegible].01	3588	1787	40.5
3	164	68.8	1.75	2603	1487	37.8
4	170	74.4	1.89	3067	1624	39.6
5	163	59.8	1.64	2487	1515	41.0
6	178	68.9	1.86	2935	1579	42.6
7	169	94.7	2.05	3549	1737	37.5
8	172	81.7	1.64	2957	1517	36.2
9	179	93.5	2.1	3484	1640	37.3
10	180	100.5	2.20	3950	1796	39.3
11	19[illegible]	93.0	2.[illegible]3	3485	1563	37.5
1[illegible]	178	96.3	2.14	3761	1759	39.1
Mean	173.9	83.0	1.95	3231	1639	39.[illegible]
±S.D.	7.9	13.2	0.[illegible]1	457	111	2.0
Coefficient of variation					6.7	5.1

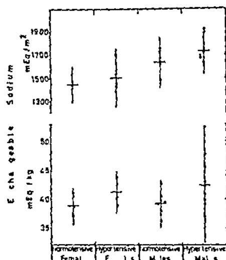


Fig 2 The relation of exchangeable sodium to weight and surface area in normotensive and hypertensive men and women The horizontal line represents the mean value for all patients the vertical line gives ±2 S D

controls Harris and Gibson (13) found that blood volume was normal and Rusznyak (21) that it was reduced in essential hypertension These investigators however compared the values obtained in hypertensives with theoretical values derived from a nomogram Reilly et al (18) studied 17 men and women with various cardiac diseases without signs of incompensation Eight of these patients were hypertensive They found a significantly lower blood volume in all 17 patients compared with a control group of 89 men (Table V) however no information was given on the height and weight of the subjects studied Rochlin et al (19) also observed a significantly lower blood volume in patients with essential hypertension compared with controls (Table V) but the hypertensive women weighed on the average 5 kg more than the controls in spite of similar height and the hypertensive men weighed 2 kg more and were 2 5 cm shorter than the average for the controls On the whole the hypertensive patients reported on by Rochlin et al were more obese than the controls which could at least in part explain the lower blood volume in the hypertensive patients Walser et al (28) found an average blood volume of 58 4 ml/kg in a group of hypertensive men and women as compared with 63 1 ml/kg in control patients (Table V) These investigators concluded however that the difference was not significant. Their hypertensive patients were also more obese than the controls

In the present study exchangeable sodium in both mEq/m² and mEq/kg was found to be significantly increased in hypertensive men Exchangeable sodium in mEq/kg was likewise increased in hypertensive women but as mentioned above direct comparison between the two groups of women hypertensives and controls is difficult because of differences in average height and weight Similar results were reported by Tominaga (27) who found that average exchangeable sodium was 46 8 mEq/kg in 36 hypertensive men and 37 2 mEq/kg in seven normotensive controls Corresponding values were 45 1 and 38 0 respectively in 15 hypertensive women and in three controls Moreover this author reported that exchangeable sodium increased with the increasing severity of hypertension Ross (20) found that average exchangeable sodium was 43 7 mEq/kg in a group of hypertensive men and women with grade I to II eyegrounds as against 38 1 mEq/kg in normals In severe hypertension with grade III to IV eyegrounds the values for exchangeable sodium were much higher Dahl et al (6) demonstrated a larger sodium pool and increased biological half life for ^{22}Na in hypertensive patients compared with controls On the other hand De Graeff (7) and Moore et al (17) observed no difference in exchangeable sodium between hypertensives and normotensives It is not possible to explain this discrepancy It is a well known fact however that sodium plays an important role in the pathogenesis of hypertension Rigid salt restriction often leads to a fall in arterial blood pressure whereas too large a sodium intake produces an increase in blood pressure (6 7 15) However as emphasized by Ross (20) and Dahl (6) it is most likely that increased exchangeable sodium is a consequence rather than a cause of hypertension

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REFERENCES

1 Allen T H Peng M T Chen K P Huang T F Chang C & Fang H S Prediction of blood volume and adiposity in man from body weight and cube of height Metabolism 5 328 1956

2. Beck B. Methyldopum i behandling af hypertensio arterialis især med henblik på vædskeretention og blodvolumen Ugeskr Læg. 125 1472, 1965
3 Berlin V L Hyde G M Parson R J Lawrence J H & Port, S Blood volume of the normal female as determined with ³²P labeled red blood cells Proc Soc. exp Biol (N Y) 76 831 1951
4 Chien, S Usami, S McAllister R. L. S F F & Gregersen, M Blood volume and age Repeated measurements on normal men *after 17 years J appl* Physiol 21 581 1956
5 Cranston, W I & Brown, W Diurnal variation in *plasma volume* in normal and hypertensive subjects. Clin Sci. 25 107 1963
6 Dahl L. K., Smilay M G Silver L & Spraragen S Evidence for prolonged biological half life of ²²Na in patients with hypertension Circulat Res 10 313 1962.
7 De Graeff J Inulin space and total exchangeable sodium in patients with essential hypertension Acta med scand 156 337 1957
8 Du Bois, D & Du Bois E F A formula to estimate the approximate surface area if height and weight be *known. Arch. intern Med.* 17 863 1916
9 Edelman I S James A H., Brooks L & Moore F D Body sodium and potassium IV The normal *total* exchangeable sodium. Its measurement and magnitude Metabolism 3 530 1954
10 Hansen, J Hydrochlorothiazide in treatment of hypertension The effects on blood volume exchangeable sodium and blood pressure Acta med scand 183 317 1968
11 Hansen, J. Alpha methyl-dopa (Aldomet®) in the treatment of hypertension. The effects on blood volume exchangeable *sodium body* weight and blood pressure Acta med scand 183 3_3 1968
12 Hansen, J & Rønnov Jessen, V Whole body hematocrit large vessel hematocrit ratio in hypertension The effect of hypotensive drugs. Acta med scand 183 17 1968
13 *Harris* A W & Gibson, J G Clinical studies of blood volume VII Changes in blood volume in Bright's disease with or without edema, renal insufficiency or congestive heart failure and hypertension J clin Invest 18 5?7 1939
14 Hopper B J Hodges J L, Brandley B Wennesland R & Yamauchi, H Red cell plasma and blood volume in healthy women measured by radiochromium cell labeling and hematocrit. J clin Invest 41 218_ 196?
15 Huff R. L. & Feller D D Relation of circulating red cell volume to body *density* and obesity *J* clin. Invest 35 1 1956
16 Meneely G R. & Dahl L K. Electrolytes in hypertension *Effects of sodium* chloride Evidence from animal and human studies Med Clin. N Amer 45 _71 1961
17 Moore F D *Edelman, J S* Olney J M James A. H Brooks, L. & Wilsen, G Body sodium and potassium III Inter related trends in alimentary *renal* and cardiovascular disease Lack of correlation between body stores and plasma concentration. Metabolism 3 334 1954
18 Reilly W A. French R M Lau F Y K Scott K. G & White W E Whole blood volume determined by radiochromium tagged red cells Comparative studies on normal and congestive heart failure patients Circulation 9 571 1954
19 Rochlin B Shohl T & Blakemore W S Blood *volume changes associated with essential* hypertension Surg Gynec Obstet 111 569 1960
20 Ross E J. Total exchangeable sodium in hypertension *Clin. Sci 15 81 1956*
21 Rusznyák S Untersuchungen zur Frage der Gesamtblutmenge des Menschen unter normalen und pathologischen Verhaltnissen Dtsch. Arch klin Med 158 98 1928
22 Rønnov Jessen, V Blood volume and *tolerance to* Pentolinium in the treatment of hypertension Lancet 2 669 1960
23 *Rønnov Jessen, V & Hansen, J* Total blood volume and exchangeable sodium in hypertension The effect of guanethidine and hydrochlorothiazide In preparation.
24 Scholer H Fundamental considerations on blood volume Amer Heart J 69 701 1965
25 Smith, A J Fluid retention produced by guanethidine Changes in body exchangeable sodium blood volume and creatinine clearance Circulation 31 490 1965
26 Smith, R H Normal blood volumes in men and *women over sixty years of* age as determined by a modified ⁵¹Cr method. Anesthesiology 19 75_, 1958
27 Tominaga T Total exchangeable sodium in human hypertension J Jap Soc intern Med 50 560 1961
28 Walser M., Duffy B J & Griffith, H W Body fluids in hypertension and mild heart failure J.A.M.A 160 858 1956

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PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

A Clinical Study

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Abstract Case reports of eight patients with the PNH defect are presented

The diagnostic criteria applied are discussed Ham s and Crosby s tests the sucrose haemolysis test acetyl choline esterase activity of the red cells and signs of intravascular haemolysis with emphasis on the determination of lactate dehydrogenase activity in blood and bone marrow

The diversity of the presentation of PNH is outlined One patient presented with attacks of abdominal pain for two years before diagnosis three patients had long standing pancytopenia with few clues to the diagnosis two patients presented as cases of aplastic anaemia with initially negative Ham s tests and two patients had concomitant myelofibrosis The relationship of PNH to other blood diseases is discussed and a possible connection with myelofibrosis is suggested. It thus appears that the PNH erythrocyte defect is encountered not only in the clinically classical PNH syndrome but also in a variety of pancytopenias which are difficult to classify and finally in some better defined conditions such as aplastic anaemia and myelofibrosis This could be interpreted in several ways 1 PNH is a nosological entity with an extremely wide clinical spectrum 2 PNH is not a nosological entity but erythroid cell lines carrying the PNH defect may be set up under many conditions when haemopoiesis is afflicted by a primary haematological disorder 3 PNH exists (*a*) as a primary disorder the clinically classical PNH syndrome and (*b*) as a secondary defect in a variety of other haematological disorders It is not yet possible to determine which of these possibilities, if any is the right one although at the present time the second interpretation would appear to be the most likely one

Clinical features are mentioned particularly hypercoagulability and the occurrence of thromboses An interesting aspect is that the majority of patients had an abnormal EEG The significance of this remains to be determined The multiple mechanism of anaemia in PNH is emphasized including marrow hypoplasia haemolysis iron deficiency autoimmunity and folic acid deficiency

The value of blood transfusions iron adrenocorticoid and anabolic steroids in the management of PNH is discussed

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare haemolytic anaemia due to a largely unknown erythrocyte defect In the classical form the disease is characterized by chronic intravascular haemolysis with occasional attacks of accentuated haemolysis during sleep which may result in haemoglobinuria

Excellent reviews have been given by Dacie (10) and Crosby (6) and recently reviews concerning pathogenetic aspects of the disease have been published by Hartmann and Jenkins (19) Firkin (15) and Hinz (24)

This paper contains the case histories of eight patients with the PNH defect which emphasize that the PNH defect occurs in conditions which clinically differ widely A point of particular interest is the occurrence of PNH red cells in myelofibrosis

METHODS AND CASE REPORTS

The erythrocyte acetylcholine esterase activity has been determined as described by Jørgensen (26) The normal range of activity in our laboratory is 16 0–32 2 μM/min/g haemoglobin The serum lactate dehydrogenase activity has been determined as described by Laursen (28) the normal range of activity being 7–23 μM/h/μl Lactate dehydrogenase isoenzymes have been determined as described by Wieme (34) and van der Helm (22) and the bands of activity have been designated 1–5 the most anodic having the lowest number Coagulation tests have been carried out in the Coagulation Laboratory as described in the following article (1) The sucrose haemolysis test was carried out as described in a following paper (18) The normal range for MCV in our laboratory is 81–109

Patient no 1 (RH A 18 526)

Farmer born in 1911 There was no family history of blood disease nor was there any exposure to drugs or chemicals except as is customary in agriculture Previ

ously the patient had been well In 1958 he began experiencing attacks of abdominal pain The attacks never started during sleep and were never accompanied by the voiding of dark or red urine At the local hospital no cause of the abdominal pain was found, and during the next two years the patient often received morphine for relief of pain In 1960 haemoglobinuria was found in connection with an attack of pain, and the diagnosis of PNH was made

In 1962 the patient was admitted to this department. Physical examination was normal Haemoglobin was 9.5 g/100 ml leucocytes and thrombocytes were normal Reticulocytes were 2.2 plasma haptoglobin 0 plasma haemoglobin 28 mg/100 ml and serum bilirubin 1.1–1.6 mg/100 ml There was intermittent haemoglobinuria, Ham's test was positive Crosby's test negative Coombs direct test Donath Landsteiner's test and a Wassermann test were all negative Osmotic fragility was slightly increased, a blood smear revealed no sphaero- or elliptocytes Haemoglobin electrophoresis was normal Serum iron was 27 μg/100 ml and TIBC 353 μg 100 ml MCV 96 MCHC 29 Roentgenologic studies of the intestinal tract were normal there was no blood in the stools and no sign of liver disease

A diagnosis of PNH with secondary iron deficiency anaemia was made and the patient was treated with oral iron, following which the haemoglobin concentration rose to 11.1 g/100 ml.

The patient continued to have attacks of severe abdominal pain Treatment at home with low molecular weight dextran had no effect The patient was last seen in this department in September 1966 As before the patient had slight haemolytic anaemia with a positive Ham's test The sucrose haemolysis test was positive serum LDH was 106 units (four times the normal value) with raised values for isoenzymes 1 and 2 Plasma haemoglobin was raised, with accentuated values during sleep Acetylcholine esterase in the erythrocytes was slightly decreased (15.4 units)

In the hope of demonstrating a hypercoagulable state as a background to the repeated attacks of abdominal pain, coagulation tests were made The tests performed at a time of no abdominal pain, were normal except for slightly increased thrombin generation One night however the patient experienced the usual abdominal pain —the first time in the eight year course of his disease when the pain occurred during sleep—and haemoglobinuria was found Coagulation tests during this attack revealed a state of pronounced hypercoagulability with short coagulation time and accelerated thromboplastin generation. This state of hypercoagulability lasted for about two days after which the state of coagulation again became as shown by the first tests. The patient was put on treatment with phenprocumonum (Marcoumar®) in the hope of reducing the severity of the abdominal pain and was discharged from the hospital This treatment, however had no effect. The patient is reported (June 1967) to suffer from unchanged attacks of abdominal pain as before

Summary Middle aged man who for two years had attacks of abdominal pain before the diagnosis of PNH was made The patient developed secondary iron deficiency anaemia which responded well to iron medication During an attack of abdominal pain a hypercoagulable state was demonstrated

Patient no 2 (RH A 21 651)

Woman, labourer born in 1909 The patient's aunt was reported to have suffered from a blood disease treated with a liver preparation but otherwise there was no family history of blood disease No known exposure to drugs or chemicals Except for fatigue from which she had suffered since childhood she had been well until 1940 when she one day experienced pain in the abdomen and voided red urine Since then the patient has never experienced similar attacks In the same year she was admitted to hospital because of pancytopenia and haemolytic anaemia and in the period 1940–65 she was hospitalized several times for the same reason Her complaints were those of tiredness and nervousness On every occasion pancytopenia and haemolytic anaemia were found but the aetiology was never clarified Ham's and Crosby's tests were not made

In January 1965 she had a sudden attack of paresis of the right extremities difficulty in speech and inability to walk An electroencephalogram was abnormal with spikes and some 6–7 Hz activity with a left sided predominance The patient quickly recovered but because of pancytopenia she was transferred to the present department in February 1965

Physical examination was normal Haemoglobin was 9 g 100 ml leucocytes 3000/μl and thrombocytes 167 000/μl Reticulocytes were 4.8–4.6% in the absence of demonstrable haemorrhage The bone marrow was hyperplastic with stainable iron Serum iron 94 μg/100 ml, TIBC 234 μg 100 ml, MCV 106 MCHC 3 Haptoglobin 4 mg/100 ml. Serum folic acid and serum B-12 were normal Ham's test was positive Crosby's test negative the acetylcholine esterase activity in the erythrocytes low (13.5 units) Coombs direct test Donath Landsteiner's test a Wassermann test osmotic fragility and haemoglobin electrophoresis were all normal An autosurvival study with chromium-51 tagged erythrocytes showed a half life of 20 days with no evidence of a double population. Ferrokinetic studies with radioisotopes indicated ineffective erythropoiesis (plasma iron clearance, half time 36 min plasma iron turnover 1.28 mg/24 h, Fe^{59} red cell incorporation at 11 days 58) Treatment with prednisone 15 mg daily was initiated but as no effect was seen after one month, prednisone was again discontinued

Since the initial admission to this department the patient has been seen on several occasions The haemolytic anaemia remains unchanged and there is still leucopenia but no thrombopenia. The patient's complaints are extreme tiredness feeling of heaviness in the right side of the body substernal oppression, and occasional right abdominal pain She has not noticed the voiding of dark or red urine but haemoglobinuria has been found on several occasions She was last seen in May 1967 The haematological parameters were unchanged except that

now some evidence of iron deficiency was found in contrast to previous studies stainable iron could no longer be demonstrated in the bone marrow The MCV had decreased from 106 to 99 although the MCHC was still normal Serum iron and TIBC were both low be cause of concomitant infection. The sucrose haemolysis test was positive serum LDH 100 with raised values for isoenzymes 1 and 2 plasma haemoglobin 10 mg/100 ml with in reased values during sleep Coagulation tests were normal. The EEG was unchanged

Summary Middle aged woman who for at least 25 years has had haemolytic anaemia and pan cytopenia of unknown aetiology The period of disease apparently started with an attack of ab dominal pain and the voiding of red urine but ex ept for this attack similar events have never occurred In 1965 the patient had an apoplectic insult, and in the same year the PNH diagnosis was made Treatment with prednisone was of no effect. Signs of iron deficiency developed

Patient no 3 (RH A 20 139)

Housewife, born in 1885 There was no family history of blood disease nor any known exposure to drugs or chemicals. Except for heavy otosclerosis she had previ ously been well

Already in 1947 pancytopenia was found During a stay at a local hosp tal to which the patient was ad mitted because of dyspnoea and cutaneous bleeding the follow ng values were found haemoglobin 31 leuco- cytes 1900/μl thrombocytes 4900/μl reticulocytes 1–3 colour index 1.3–1 6 ESR 44–83 mm/h Haemo glob nuria was not demonstrated No cause of the pan cytopen a was found and treatment with folic acid and a liver preparation was without effect. Several transfusions with whole blood were given After one of them un explained fever developed During the following years the patient apparently did well and was not admitted to hosp tal In 1961 she was admitted to a dermatological hospital because of a post thrombotic ulcer of the leg. Again pancytopen a was found and the patient was transferred to a med cal department of the local hospi tal Haemoglobin was 7 8 g/100 ml leucocytes 1900/μl, thrombocytes 19 000/μl and reticulocytes 1 5–2 0% For the first time during her illness Ham s and Crosby s tests were made both were slightly pos tive There was still no haemoglobinuria.

In 1963 the patient was admitted to the present de- partment Her complaints were still only those attribut able to anaemia Except for obesity and a chronic ulcer of the leg, physical examination was normal. Haemo- glob n was 8.2 g/100 ml leucocytes 1400/μl thrombo- cytes 64 000/μl The anaemia was haemolytic reticulo- cytes 2 0–3 0 % with no signs of bleeding or blood rege neration, serum haptoglobin 0 plasma haemoglobin 7–10 mg/100 ml An erythrocyte autosurvival study with chromium 51 tagged cells showed a half life of 19 ½ days with no evidence of a double erythrocyte popula tion. Ham s test was pos tive Crosby s test negative haemoglobinuria could not be demonstrated Coombs direct test, Donath Landsteiner s test and a Wassermann test were all negative Osmotic fragility was normal a blood smear revealed no sphaero- or elliptocytes Serum iron was 59 μg/100 ml TIBC 268 μg/100 ml MCV 107 MCHC 30

A diagnosis of atypical PNH was made Treatment with prednisone 20 mg a day was initiated and the pa tient was discharged for further control in the outpatient department The patient however did not show up until one year later when she was readmitted because of ulcer of the leg During this year the patient had continuously taken the prescribed prednisone On this treatment haemoglobin had risen to 1? 1 g/100 ml and there was only slight reticulocytosis (<1%) Leucocytes were 3000 and thrombocytes 90 000/μl Ham s and Crosby s tests were now negative The patient felt com pletely well and she was discharged on prednisone 10 mg daily Unfortunately it proved impossible to keep con tact with the pati nt but she continued to take the prednisone as prescribed In 1966 she was admitted to another hospital because of gangrene of a leg From the records of the hospital it appears that the haematological values were still higher than the values prior to the initiation of prednisone treatment (haemoglobin 10 1 g/100 ml leucocytes 3300–6 00/μl thrombocytes 1 8 000–113 000/μl) She died in 1966 of acute renal failure following amputation of a leg Renal venous thrombosis was suspected but no autopsy was per formed

Summary Old woman who for at least 19 years had pancy openia and haemolytic anaemia Ham s test was not performed until 1961 14 years after pancytopenia had first been demonstrated and was then positive Ham s test was positive also in 1963 but in 1964—after one year of prednisone treatment—it had become negative while the haemoglobin concentration had risen to almost normal levels where it remained until her death in 1966 The patient s complaints were only those of anaemia Haemoglobinuria was never demon strated

Patient no 4 (RH A.22 655)

Housewife born in 19?6 There was no family history of blood disease nor any known exposure to drugs or chemicals.

Since childhood the patient has had a tendency to anaemia, for which she has often been treated with iron. In 1955 1956 and 1959 she was admitted to hospital be cause of anaemia On all these occasions pancytopenia and haemolyt c anaemia were found but the aetiology was never el cited Ham s and Crosby s tests were not performed The pat ent s complaints were those of anaemia and dermal infections Treatment with vitamin B_{12} and folic acid had no effect.

In May 1966 the patient like the rest of her family suddenly fell ill with fever nausea, vomiting and dizzi- ness The rest of the family quickly recovered but three

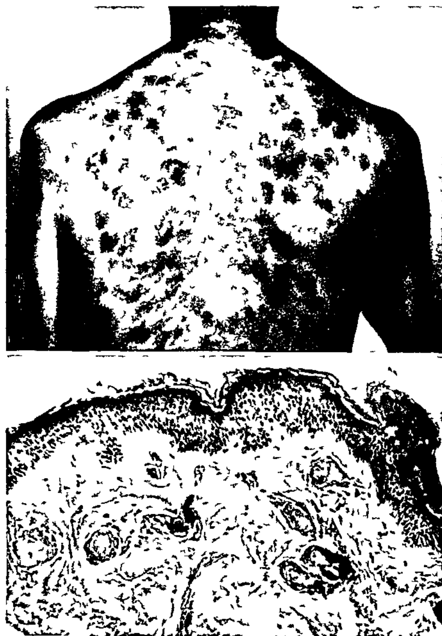


Fig 1 Patient no 4 Affected skin and corresponding biopsy with thromboses in capillaries and arterioles suggestive of thrombotic thrombocytopenic purpura

days after the onset of the disease the patient developed red and blue spots universally on the skin and shortly afterwards pain in the joints and muscles Treatment with Ampicillin had no effect, and therefore the patient was admitted to the present hospital

Physical examination was normal except that her skin was covered with reddish blue spots (Fig 1) The temperature was around 39 C Haemoglobin was 8 1 g/100 ml leucocytes ?200/μl and thrombocytes 65 000/μl Reticulocytes were 1.2–2 1% Although no bleeding could be demonstrated the haemoglobin concentration fell from 8 1 to 6 6 in four days The bone marrow was hyperplastic with no stainable iron Serum iron 61 μg/100 ml TIBC 335 μg/100 ml MCV 1?6 MCHC 33

There was haemosiderinuria but no haemoglobinuria Ham s test was positive Crosby s test negative The acetylcholine esterase in the erythrocytes was low (10.3 units) A direct Coombs test was slightly positive and the antinuclear factor test was ++ Donath Landsteiner s test and a Wassermann test were negative Osmotic fragility was slightly increased

A biopsy from the affected skin showed changes compatible with thrombotic thrombocytopenic purpura (Fig 1) There was however no sign of renal involvement, and neurological examination was normal An electroencephalogram was moderately abnormal with diffuse admixture of 3–5 Hz activity Coagulation tests showed pronounced hypercoagulability with shortened coagula-

tion time accelerated thrombin generation and in creased concentrations of factors II + VII V and VIII

The patient was considered to suffer from PNH with acute immunological complications, and on this assumption treatment with prednisone 80 mg daily was initiated The response was striking the patient became afebrile the haemoglobin leucocytes and thrombocytes rose to normal values the Coombs test became negative and the cutaneous changes disappeared The patient was discharged on oral iron and prednisone 15 mg daily

The patient wa again admitted to this department in December 1966 for a check up She had no complaints at all and reported that she felt better than during the past 15 years Haemoglobin, leucocyte and thrombocyte values were normal there were still signs of haemolysis with reticulocytes 6 6–6 8% and a serum LDH of 58 units (twice the normal upper value) the isoenzyme pattern was compatible with haemolysis (raised values for isoenzymes 1 and 2) Plasma haemoglobin was 10 mg/100 ml with increases during physical activity but not during sleep The concentrations of coagulation factors were increased as during her first admission Skin lesions were absent The patient is still well and in complete haematological remission on a moderate dose of prednisone and oral iron She has never had attacks of abdominal pain with red or dark urine and haemoglobinuria has never been demonstrated

Summary Middle aged woman who for at least 11 years has had pancytopenia and haemolytic anaemia In connection with a febrile disease the patient developed skin lesions histologically compatible with thrombotic thrombocytopenic purpura and a transiently positive Coombs test At the same time evidence of a PNH defect was found On treatment with prednisone the symptoms vanished and complete haematological remission developed Haemolytic anaemia with a positive Ham s test persists but clinically the patient is completely well

Patient no 5 (RH A 18 497)

Girl born in 1949 There is no family history of blood diseases

During the period 1952–1957 the patient was treated with large amounts of penicillin because of dermal infections Since the age of four the patient was often treated with iron because of recurrent anaemia and she had a pronounced tendency to develop blue marks in the skin and nose bleeding. In 1955 the patient had a concussion of the brain and since then has had frequent attacks of headache of the hemicrania type for which she has been treated with dehydroergotamine

In January 1963 the patient suddenly developed high fever of unknown aetiology Two days later petecchiae developed and the patient was admitted to the local hospital Pancytopenia with a hypoplastic bone marrow was found On the diagnosis of aplastic anaemia treatment was instituted with prednisone metandienone (Dianabol®) and vitamin B_{12}. The leucocyte count rapidly became normal but the haemoglobin and the thrombocyte count did not improve and therefore the patient was admitted to this department in May 1963

Physical examination was normal Haemoglobin was 8 6 g/100 ml thrombocytes 32 000/μl, the leucocyte count normal Reticulocytes were 3 0 serum bilirubin 1 1 mg/100 ml No bleeding could be demonstrated Ham s and Crosby s tests were negative and there was no haemoglobinuria Coombs direct test Donath Landsteiner s test and a Wassermann test were all negative Osmotic fragility was slightly increased There were no sphaero- or elliptocytes in the blood smear Haemoglobin electrophoresis was normal

A diagnosis of hypoplastic anaemia was made and treatment with prednisone 25 mg daily and nortestosterone 25 mg a week was given. There was no striking response to this treatment and as severe thrombocytopenia continued splenectomy was performed in October 1964 The spleen weighed 150 g and was microscopically normal After splenectomy the haemoglobin concentration rose to a higher level than before the operation but the thrombopenia was unchanged As the patient became strongly cushingoid prednisone was discontinued but this was followed by a decrease in haemoglobin from 10 9 to 7 4 g/100 ml in one month Therefore prednisone treatment was resumed after which the haemoglobin concentration increased again The thrombocyte count was continuously low and intermittently haemorrhagic diathesis, often as haematuria was present Ham s and Crosby s tests were still negative

In March 1966 the patient was readmitted because of decreasing haemoglobin concentration The haemoglobin was 8 g/100 ml thrombocytes 42 000/μl leucocytes normal Reticulocytes were 4 0% For the first time during her illness Ham s and Crosby s tests were positive Haemosiderinuria but no haemoglobinuria was present The acetylcholine esterase activity in the erythrocytes was low (13.5 units) Her symptoms were still only those of anaemia and thrombocytopenia The dose of prednisone was increased but the haemoglobin concentration continued to fall Six months later the patient was readmitted She had now developed the full picture of PNH attacks of abdominal pain accompanied by haemoglobinuria and chronic intravascular haemolysis Ham s test was positive Crosby s test negative the sucrose haemolys s test positive Plasma haemoglobin was only 2 mg per 100 ml but with accentuated values during sleep Serum LDH was 98 units with raised values for isoenzymes 1 and 2 Coagulation tests showed accelerated thrombin generation and thromboplastin generation and short cephalin time During a haemolytic attack the patient developed an accentuated degree of hypercoagulability with an accelerated thromboplastin generation time short cephalin time and now also raised values for factors V and VIII During sleep the EEG was normal but upon photo-stimulation universal spikes were seen Haemoglobin decreased to 6 1 g/100 ml Prednisone which earlier in the disease had been given with effect was increased to 40 mg daily but the haemoglobin concentration was unaffected At this time the serum iron was only 1–6/100 ml in spite of severe haemolysis TIBC 345 μg/100 ml MCV 1 6 MCHC 29 and there were few

sideroblasts in the marrow Iron deficiency was suspected and the patient was put on oral iron medication while at the same time the prednisone dose was reduced gradually Over the next few months this resulted in an increasing haemoglobin concentration In the middle of May 1967 the haemoglobin concentration was 9 6 g/100 ml the MCHC 31 and the patient still seems to be improving

Summary Young girl who in 1962 developed aplastic anaemia Treatment with prednisone anabolic steroid and splenectomy resulted in partial remission though the thrombocytopenia was largely unaffected In 1966 the haemoglobin concentration decreased considerably and at this time Ham s test—negative at the onset of disease—became positive Six months later the patient had developed the full picture of PNH Iron deficiency developed and medication with oral iron resulted in a significant increase of haemoglobin

Patient no 6 (RH A 20 050)

Farmer born in 1928 There was no history of blood disease in the family nor was there any exposure to drugs or chemicals except as is customary in agriculture The patient had been well until the present illness started in October 1962 with symptoms of anaemia and cutaneous haemorrhages At the local hospital pancytopenia was found the bone marrow was hypoplastic On the diagnosis of aplastic anaemia treatment with prednisone 30 mg daily was started and several transfusions with whole blood were given

The patient was first admitted to this department in January 1963 Physical examination was normal Haemoglobin was 5 7 g 100 ml leucocytes 1700–1900/μl, thrombocytes 54 000–19 000 μl, and reticulocytes 1–1 6 A biopsy from the iliac crest showed a hypoplastic marrow An autosurvival study with chromium 51 erythrocytes demonstrated a half life of 16 days without evidence of a double population Serum iron 232 μg TIBC 267 μg 100 ml MCV 105 MCHC 35 Ham s and Crosby s tests were negative and there was no haemoglobinuria Coombs direct test Donath Landsteiner s test and a Wassermann test were all negative

The diagnosis of aplastic anaemia was confirmed and treatment with metandienone (Dianabol®) 10 mg daily and prednisone 30 mg a day was given On this treatment the haemoglobin became normal and the thrombocyte count rose to 90 000/μl This remission lasted for about one year Then the haemoglobin concentration fell again and the patient was readmitted in September 1964

The haemoglobin now was 8 g 100 ml The bone marrow was hyperplastic with definite megaloblastic features The serum folic acid concentration was decreased (0 003 μg/ml) whereas serum B_{12} was normal Folic acid was now given together with Dianabol and prednisone Haemoglobin values quickly began to rise and six months later the haemoglobin was normal

One year later the haematological values decreased again and the patient was readmitted in December 1965 Except for fatigue the patient had no complaints Haemoglobin was 6 5 g/100 ml thrombocytes 45 000 and leucocytes 3200/μl Reticulocytes were 2 4 The marrow showed activity both in the erythro- and granulocytopoiesis The half life of the patient s erythrocytes tagged with *chromium 51* was 20 days without evidence of a *double population* Ham s test was now positive Crosby s test questionably positive There was neither haemoglobinuria *nor* haemosiderinuria Donath Landsteiner s test a Wassermann test and a direct Coombs test were all negative The patient was discharged on prednisone 30 mg a day Dianabol and folic acid as before

Again haemoglobin rose to normal thrombocytes were 100 000/μl leucocyte count normal The patient felt completely well and worked full time as a truck driver The patient was last seen in January 1967 Haemoglobin thrombocytes and leucocytes were normal there was no haemoglobinuria and no haemosiderinuria Ham s test was slightly positive whereas Crosby s test now was negative The sucrose haemolysis test was positive Plasma haemoglobin was 5 mg per 100 ml with a moderate increase during sleep Serum (LDH) was 23 units (i e upper normal limit) with raised values for isoenzymes 1 and 2 The acetylcholine esterase activity in the erythrocytes was normal The patient was in a hypercoagulable state with increased concentrations of factors II and VII V and VIII and a slightly accelerated thromboplastin generation An electroencephalogram was normal The patient was discharged on prednisone 12.5 mg Dianabol and folic acid as before and is at present still in complete haematological remission

Summary A 36 year-old farmer who developed aplastic anaemia of unknown aetiology Treatment with prednisone and anabolic hormone resulted in normal haemoglobin and raised the leucocyte and thrombocyte counts Next he developed secondary folic acid deficiency with depressed blood counts After folic acid medication the haemoglobin again became normal At the onset of the disease Ham s test was negative but became positive four years later concomitantly with a relapse of anaemia Haemoglobinuria and haemosiderinuria have never been found and now—five years after the start of the disease and more than one year after the Ham s test became positive—the patient is completely well with only serological signs of his disease Treatment with prednisone and anabolic steroid seems to have been beneficial during the whole course of the disease

Patient no 7 (RH A 22 939)

Ships captain born in 1917 There was no family history of blood disease and no known exposure to drugs or chemicals In 1935 the patient had had a single attack

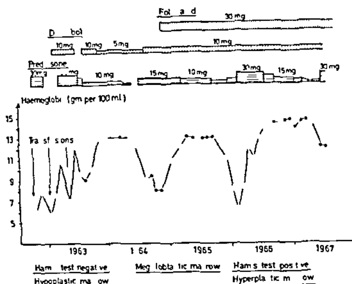


Fig 2 Development of haemoglobin concentration during the course from hypoplastic anaemia to PNH and the treatment given (patient no 6)

of malaria. Except for this and several uncomplicated gonorrhoeal infections he had previously been well

In 1958 the patient began to suffer from attacks of low back pain accompanied by voiding of dark urine He felt reasonably well however until the summer of 1964 when he experienced pain in the feet which turned bluish in colour In December 1964 he was admitted to the local hospital where he was followed during the next years Physical examination revealed a moderate hepatomegaly Haemoglobin was 70–50% leucocytes 10–40 000/μl thrombocytes 500 000–1 300 000/μl and reticulocytes 2–10% In the beginning Coombs direct test was slightly positive but soon became negative Ham s and Crosby s tests were not performed The urine was reported to contain haemoglobin on several occasions and periodically the patient was jaundiced with no other signs of liver disease On the assumption that the anaemia was due to autoimmune haemolysis treatment with prednisone 40–15 mg daily was given There was no striking response to this treatment and several transfusions of whole blood were given to the patient without any ill effect In March 1966 an infiltrate in the apex of the right lung was first seen on the chest X ray The nature of this infiltrate could not be determined

In November 1966 the patient was admitted to this department He complained of fatigue and attacks of slight fever accompanied by low back pain and the voiding of dark urine The previously mentioned pain in the feet persisted. Physical examination revealed a chronically sick man with the liver extending 5 cm below the right costal margin and the spleen palpable 2 cm below the left costal margin The skin over the right ankle was bluish in colour The haemoglobin was 7 9–5 3 g 100 ml leucocytes ?0 000/μl (65% granulocytes 2% band forms, 1% metamyelocytes, 1 myelocytes 8% monocytes ?5% lymphocytes, 1% normoblasts) thrombocytes 850 000–1 0?4 000/μl (in part giant platelets) and reticulocytes 14 6–29.2% Bleeding could not be demonstrated MCV 89 MCHC 30 serum iron 90 μg/100 ml and TIBC 214 μg/100 ml The bone marrow showed marked erythroid and granulopoietic hyperplasia with a normal number of sideroblasts The Ph chromosome was not present Plasma haemoglobin wa 23 mg/100 ml with accentuated values during sleep there was haemosiderinuria and occasional haemoglobinuria serum haptoglobin O Serum LDH was 230 units (ten times the normal value) with an isoenzyme pattern compatible with haemolysis (raised values for isoenzymes 1 and ?) Serum bilirubin 1 4–1.5 mg/100 ml with no other laboratory signs of liver disease Ham s test was positive Crosby s negative the sucrose haemolysis test positive The acetylcholine esterase activity in the erythrocytes was decreased (13 8 units) Coombs direct test Donath Landsteiner s test and a Wassermann test were all negative A haemoglobin electrophoresis was normal Malaria parasites could not be demonstrated in the blood Osmotic fragility was slightly increased Ferrokinetic studies with radio iron revealed an effective increased erythropoiesis. An autosurvival study with chromium 51 tagged erythrocytes yielded two slopes the first indicating a half life of 10 5 days the other a half life of 21 days The change in slope of the survival curve coincided with the start of prednisone treatment. Except for the thrombocytosis, coagulation tests were normal An electroencephalogram was abnormal with sharp-waves/spikes in the left temporo-frontal region A chest X ray revealed that the infiltrate in the apex of the right lung persisted The nature of the infiltrate could still not be determined Examination of the sputum for tubercle bacili and malignant cells was negative

The diagnosis was uncertain There was good evidence of a PNH defect, but the hepato-splenomegaly and the leuco- and thrombocytosis were not compatible with the conventional picture of PNH The most likely diagnosis covering these features would be early myelofibrosis and it was therefore assumed that the patient suffered from early myelofibrosis associated with a PNH defect The possibility that the leuco- and thrombocytosis and the

hepato-splenomegaly could be caused by a malignant tumour in the lung with metastases was not considered very likely in view of the fact that these features had been present at least two years prior to the development of the pulmonary infiltrate The pain in the feet and the bluish colour of the skin over the ankle were considered to be small thrombotic manifestations of the heavy thrombocytosis

Treatment with prednisone 40 mg daily and busulfan (Myleran®) was given Antituberculous treatment was also instituted because of the indeterminate nature of the lung infiltrate In addition, several transfusions with washed erythrocytes were given. On this treatment the thrombocytes and leucocytes fell to normal values It was difficult to evaluate the effect of prednisone on the anaemia, but there appeared to be a moderate effect

The patient was discharged in December 1966 and readmitted in January 1967 During this period palpable lymph nodes had developed on the right side of the neck A biopsy from these nodes showed metastatic car cinoma The site of the primary tumour could not be determined with certainty but the histological picture was compatible with a primary bronchogenic carcinoma This diagnosis found further support from the fact that the infiltrate in the apex of the right lung had grown further and the mediastinum had increased in width Oedema of the neck developed but regressed somewhat when radiotherapy to the mediastinal mass was given The patient was discharged in February 1967 on predni sone 25 mg daily In March 1967 the patient died sud denly at home No autopsy was performed

Summary Middle aged man who since 1958 had attacks of low back pain accompanied by the voiding of dark urine In 1964 hepato spleno megaly thrombo and leucocytosis and a haemo lytic anaemia which in the beginning was Coombs positive were found In 1966 good evidence of the PNH defect was observed and the patient was considered to suffer from early myelofibrosis associated with a PNH defect In 1966 a tumour in the right lung developed probably a bronchogenic carcinoma The patient died suddenly in March 1967 No autopsy was performed

Patient no 8 (RH A 22 104)

Male civil servant born in 1910 The patients mother was reported to have had pernicious anaemia, but other wise no family history of blood diseases could be elic-ited. There was no known exposure to drugs or chemi cals In 1926 the patient had jaundice probably due to epidemic hepatitis In 1933 the patient had haematuria of unexplained aetiology Otherwise he had previously been well

During the spring of 1965 the patient felt progressively tired lost weight and sweated profusely He was ad mitted to this department in September 1965 Physical examination revealed considerable enlargement of the spleen and liver There was a haemoglobin of 9.5 g/100 ml leucocytes 10 400–11 100/μl and thrombocytes 219 000–280 000/μl A differential count showed 67% granulocytes 16% band forms 2 metamyelocytes 3% myelocytes 6% eosinophils 4% lymphocytes 2 mono-cytes 2% myeloblasts and 5 normoblasts Reticulo-cytes were 6 7–7 6 serum haptoglobin 48 mg/100 ml An autosurvival curve with chromium 51 tagged erythro-cytes showed a half life of 15 days with no evidence of a double population. No occult bleeding could be de monstrated Serum iron was 57 μg TIBC 347 μg/100 ml MCV 10_ MCHC 31 A biopsy from the iliac crest revealed a strongly hyperplastic marrow with reticulum cells and many megacaryocytes but no fibrosis Liver function tests were normal The basal metabolic rate was 149

On the diagnosis of early myelofibrosis treatment with prednisone 30 mg a day was initiated and later metan dienone (Dianabol®) 10 mg daily was added to the medication As haemoglobin fell to 7–8 g/100 ml and the hepato splenomegaly increased X ray therapy was given to the spleen 500 r were given but the size of the spleen remained unchanged and the haemoglobin concentration fell further to 6 g/100 ml The patient was readmitted in February 1967

There was pronounced haemolysis (reticulocytes ?7–35) a slight leucocytosis and normal thrombocyte counts During the past month the patient had noticed dark urine on rising in the morning and as haemo-globinuria was now found, tests relevant to PNH were performed Ham s test was positive Crosby s test nega tive the sucrose haemolysis test was positive The acetyl choline esterase activity in the erythrocytes was 18.5 units (normal) just after an attack of haemoglobinuria but determination of the enzyme activity performed at the time of no overt haemoglobinuria showed low values (13–14 units) Serum LDH was 257 units with an iso-enzyme pattern suggestive of haemolysis (raised values for isoenzymes 1 and 2) Plasma haemoglobin was 15 mg/100 ml A new autosurvival study with chromium 51 tagged erythrocytes revealed two populations one with a half life of 7 days, the other with a half life of 16 days. There was a definite accumulation of activity over the spleen. Donath Landsteiner s test a Wassermann test and Coombs direct test were all negative Osmotic fragility was slightly increased Serum iron was 57 TIBC 269 μg/100 ml MCV 113 MCHC 27 There was no stainable iron in the marrow Coagulation tests revealed hyper coagulability with raised thrombin values and accelerated thromboplastin generation An electroencephalogram dis-played a rather low dominant frequency (8 Hz) but otherwise was normal The patient was now considered to suffer from concomitant myelofibrosis and PNH Be cause of the suspected iron deficiency which might contribute to the pronounced anaemia oral iron was given for a short period with no discernible effect Be cause there was reason to believe that the spleen played a major role in the genesis of the severe haemolytic anaemia the patient was submitted to splenectomy in March 1967

The operation was uncomplicated The spleen weighed 1800 g and showed marked myeloid metaplasia but no

fibrosis The postoperative period was stormy Immediately after the operation two major complications developed severe thrombocytosis (thrombocyte counts around 1,500 000/μl) and anuria, the latter probably caused by haemoglobinuria precipitated by the infusion of concentrated plasma proteins given in the surgical department In order to diminish the thrombocytosis and stop the haemolysis with the continuous load of haemoglobin on the kidneys thrombocytopheresis and exchange transfusions with washed donor erythrocytes were carried out. The thrombocytopheresis was done continuously over a period of three days and the exchange transfusions continuously over a 24-hour period At the same time peritoneal dialysis was done Nitrogen mustard was given to restrain platelet production The result of this intensive treatment was encouraging the thrombocyte count decreased to acceptable levels, and the haemolysis was virtually stopped as evidenced by the disapperance of haemoglobin in the plasma and a fall in the reticulocyte count to almost nil. During the following months, however further complications occurred high fever probably due to a pulmonary infection, a hepatic disease probably inoculation hepatitis, recurrence of thrombocytosis which necessitated nitrogen mustard and busulfan therapy and deep thromboses of both legs at a time when the platelet count was only moderately elevated and the patient was ambulatory This in turn was followed by spiking fever which, at least in part was explained by a small abscess in the abdominal wall. Transfusions with washed erythrocytes had to be given at intervals. Because of these numerous complications an evaluation of the possible effect of the splenectomy can not be made at the present time In the months following the operation the only evidence of the PNH defect was a slightly positive sucrose haemolysis test Haemosiderinuria was constantly present. Ham s and Crosby s tests were negative and the acetylcholine esterase activity in the erythrocytes was normal It must be borne in mind that a fair part of the erythrocytes at this time were of donor origin It is also likely that the many transfus ons with washed erythrocytes have suppressed the production of erythrocytes with the PNH defect

Summary Middle aged man with probable myelofibrosis (hepato-splenomegaly with myeloid metaplasia leuco-erythroblastic haemolytic anaemia slight leucocytosis many megakaryocytes and reticulum cells in the marrow and an elevated basal metabolic rate) Eighteen months after the onset of the disease haemoglobinuria appeared and good evidence of the PNH defect was found After splenectomy numerous complications including thrombocytosis and anuria probably caused by a haemolytic attack occurred

DISCUSSION

The present series comprises eight patients with the PNH defect seen in this department over a period of six years It has been estimated that the incidence of PNH is two per 1 million (6) which would correspond to nine patients in Denmark at the present time The appearance of eight patients in one medical department (albeit a department with a special interest in haematology) during a relatively short period of time may indicate however that PNH or at least the PNH defect is more common than previously supposed The reason for this is probably that in recent years PNH has been looked for—and found—in patients who do not fit the classical picture of PNH and certainly not the apparently quite inappropriate name given to the disease Only one of our patients (no 1) represented a fairly typical case of PNH whereas three patients (nos 2, 3 and 4) had long-standing idiopathic pancytopenia and haemolytic anaemia, two patients masqueraded as cases of idiopathic aplastic anaemia (nos 5 and 6) and two patients were diagnosed as myelofibrosis (nos 7 and 8)

Diagnosis

Although it is recognized that PNH is sometimes seen in forms which differ from the classical description most of our patients deviate so much from the original concept of PNH that a consideration of the criteria for the diagnosis is mandatory *Ham s test (the acidified serum test)* is recognized as diagnostic of PNH if carried out with adequate controls with unacidified serum normal erythrocytes and inactivated serum (10) This test was positive in all our patients at one time or another during the course of the disease The diagnosis does not, however rest solely on this test but is supported by additional evidence as mentioned below

The thrombin haemolysis test (Crosby s test) has been very inconsistent in our patients Positive tests were encountered only in three patients (nos 3 5 and 6) The test was often negative in the sense that thrombin did not enhance and sometimes even inhibited haemolysis Several explanations of this may be imagined A rather remote possibility would be that patients with a positive Ham s test and a negative Crosby s test might constitute a special group of PNH patients At the present time there is nothing to support this hypothesis The reason for the negative outcome of the test in most of our patients is rather a technical one namely that certain commercial



Fig 3 LDH isoenzyme patterns in bone marrow (*upper*) and in peripheral blood plasma (*lower*) Anodic iso enzymes to the left

brands of thrombin contain additives which inhibit PNH haemolysis (7) A comparison of the brand used by the Statens Seruminstitut Copen hagen where the tests in our patients were per formed with other thrombin preparations is at present being made In view of this and with all the other evidence in favour of the PNH defect in our patients we do not consider that the nega tive outcome of the thrombin haemolysis test disproves the diagnosis of PNH

Recently Hartmann and Jenkins have intro duced new diagnostic tests for the PNH defect *the sugar water test* and *the sucrose haemolysis test* (21) Whereas the sugar water test varied considerably and was frequently negative the sucrose haemolysis test was positive in all the pa tients tested also when carried out in isologous compatible serum Our experience with this new diagnostic test for PNH will be further discussed in a subsequent paper (18) In our opinion the test is of value in the diagnosis of PNH It ap pears to be specific and since it is technically easy to perform it will probably become a useful screening test for the PNH defect Moreover the test may be more sensitive than Ham s test as suggested by the observations in patient no 8

As first reported by de Sandre (12) the *acetyl choline esterase activity* (ACHE) in PNH erythro cytes is low The aetiological significance of this observation is still unknown (31) Low ACHE is occasionally present in other blood diseases but only in PNH are low values found consistently Therefore determination of red cell ACHE is of definite h lp in establishing the diagnosis of PNH Whereas in normal blood the highest ACHE activity is found in reticulocyte rich fractions the opposite is true of PNH blood a finding com patible with the fact that in PNH blood the reticulocyte rich fraction contains the highest pro portion of cells susceptible to acid haemolysis (3) However certain experimental models seem to indicate that low ACHE values do not sys tematically coincide with a positive Ham s test. Consequently determination of enzyme activity may yield information about the cells which is not provided by the acidified serum test (14 23) In a series of 65 PNH patients Metz (30) found that the most severely ill patients had low values whereas normal values were found in three pa tients with only mild disease This is in keeping with our results the six patients with active disease having had low activities while the pa tient who only exhibited laboratory evidence of disease (no 6) had a normal value The estima tion of the severity of the disease from the ACHE activity does however involve one theoretical fallacy after a significant haemolytic attack the number of PNH cells in the blood may be low and consequently the ACHE activity may be normal This was observed in one patient (no 8) who had normal ACHE values just after a haemolytic attack but low activities in periods with no overt haemoglobinuria

The usual signs indicating *intravascular haemolysis* present or past i e low serum haptoglobin high plasma haemoglobin haemosiderinuria and occasional haemoglobinuria were present in vary ing degrees in our patients However these signs are not always present even in manifest haemo lysis and evaluation of the degree of haemolysis based on these parameters is not always possible The determining factor among the above men tioned sign is the haptoglobin The amount of this haemoglobin binding protein is increased during infections in malignancy and during steroid medication (5) and as one or more of these factors were present in many of our pa tients—and is likely to be in many PNH patients —the applicability of these signs in determining the degree of intravascular haemolysis is there fore correspondingly limited Similarly other classical signs of haemolysis such as reticulocy

Table I *Diagnostic criteria of the PNH diagnosis*

Pat. no	Ham's test	Crosby's test	Sucrose haemolysis test	Acetylcholine esterase in erythrocytes (normal 16 0–23 2 units)	Haemoglobinuria and/or haemosiderinuria	Plasma haemoglobin (mg/100 ml)	Serum LDH units (normal <23 units)
1	+	−	+	15 4	+	15	106
2	+	−	+	13 5	+	13	100
3	− → −	+ → −	Not done	Not done	−	Not done	Not done
4	+	−	+	10 3	−	6	58
5	+	+ → −	+	13 5	+	2	98
6	+	(+) → −	+	20	−	4	23
7	+	−	+	13 8	+	16	230
8	+	−	+	13	+	14	257

tosis in the presence of a constant or decreasing haemoglobin concentration and no detectable bleeding may not give a correct estimate of the degree of haemolysis since other factors in the PNH anaemia (e g marrow hypoplasia iron deficiency) may influence the picture

It was therefore felt that other parameters of intravascular haemolysis should also be studied determination of plasma *lactate dehydrogenase (LDH) activity* combined with *LDH isoenzyme analysis* proved to be of value These studies were carried out in seven patients The six patients with active disease all had markedly raised total LDH values (up to 10–15 times normal) whereas the one patient with inactive disease had a high borderline value (patient no 6) By comparing these value with the respective values for plasma haemoglobin (Table I) it is seen that LDH values are more closely related to the haemolytic activity in most of the patients In order to determine the origin of the elevated LDH concentrations LDH isoenzyme analysis was performed on erythrocytes and peripheral blood plasma as well as on marrow plasma and marrow nucleated cells This was done on the assumption that if intramedullary haemolysis—which is sometimes found in PNH patients and is thought to be the source of the markedly elevated LDH values in untreated pernicious anaemia—should contribute to the elevated values this would show up in the isoenzyme pattern The results are shown in Fig. 3 It is seen that in peripheral plasma isoenzymes 1 and 2 predominate corresponding closely to the erythrocyte pattern whereas in marrow plasma the pattern is dominated by isoenzymes 2 3 and 4 corresponding to the pattern in nucleated marrow cells These results of course do not answer the question of whether or not intramedullary haemolysis is present in PNH but they do seem to indicate that at least the main source of the elevated peripheral plasma values is intravascular haemolysis

In haematological disorders markedly raised LDH values are found especially in untreated pernicious anaemia whereas most haemolytic anaemias (mainly with extravascular haemolysis) only show moderately elevated values (13) It has even been considered that markedly raised values are indicative of pernicious anaemia but in our experience haemolytic anaemias with intravascular haemolysis including PNH must also be considered when particularly high concentrations are encountered

The basis for the diagnosis in this series of patients has been discussed so thoroughly because the course of the disease in most of our patients has been rather atypical If the diagnosis rested solely on a positive Ham's test, doubts as to the significance of this test in our patients could have been raised However as demonstrated the diagnosis in each case is supported by other evidence The diagnostic criteria are summarized in Table I The exact classification of some of the presented cases may be a matter of opinion but it can hardly be disputed that all of them had the PNH defect.

Relationship to other blood diseases

In the past PNH was considered a *primary* disease However during recent years it has become increasingly evident that the PNH defect sometimes develops in patients suffering from aplastic anaemia The largest series of patients with aplastic anaemia studied with respect to the

PNH defect was reported by Lewis and Dacie (29) who observed the development of the PNH defect in seven out of 46 patients originally diagnosed as cases of aplastic anaemia This sequence of events appears to be too frequent to *be incidental The relationship between the two* diseases is also supported by the somewhat similar behaviour of the red cells in the two diseases towards complement lysis as demonstrated by Rosse and Dacie (33) Dacie (9) suggests that the PNH defect develops as a complication of aplastic anaemia when haemopoietic regeneration takes place it is assumed that part of the repopulation is derived from a clone of abnormal stem cells

Two of our patients (nos 5 and 6) presented as cases of aplastic anaemia of unknown aetiology The acid haemolysis test, negative at the onset of the disease became positive after four years One of the patients (no 5) developed the full picture of PNH six months after the acid haemolysis test was first found positive In the other patient the PNH defect has till now—more than one year after acid haemolysis was first found—remained a laboratory phenomenon

During the four years prior to the first demonstration of the PNH defect both patients achieved partial or complete remission on a combined treatment of prednisone and anabolic steroid The occurrence of acid haemolysis in our patients was heralded by a drop in haemoglobin concentration The first appearance of a positive Ham s test did not concur with marrow repopulation However this is not necessarily an argument against the above mentioned theory of the development of the PNH defect Ham s test may be a crude test in the sense that it may require a considerable number of abnormal cells in order to yield a positive result or it may reveal a qualitative red cell defect which is a late event in the development of the PNH syndrome One way to obtain an earlier diagnosis of the PNH defect might be to apply Ham s test to the youngest erythrocytes *which are likely to contain the largest fraction of* abnormal cells (29) an even greater refinement may be to study the effect of acidified serum on isotope liberation from radio-iron labeled patient red cells (17) Also the recently developed complement lysis test (33) which measures the complement sensitivity of erythrocytes may prove to be a valuable tool in early diagnosis of PNH as demonstrated by one of the patients reported by Rosse and Dacie who developed a positive complemen lysis test before the acid haemolysis test became positive Similarly the sucrose haemolysis test may be sensitive enough to aid in *early diagnosis experience with this test is still* limited but observations like those in our patient no 8 may suggest that it is more sensitive than Ham s test

It is interesting that the PNH defect was found also in two patients diagnosed as cases of myelofibrosis (patients 7 and 8) Only one case with this combination has previously been reported (11) It is not likely that this is a coincidence As in aplastic anaemia one might visualize that the strain imposed upon haemopoiesis by the primary disease favours the outgrowth of an abnormal haemopoietic cell clone Since the basic lesion in myelofibrosis is believed to be a differentiation disturbance of a pluripotential stem cell one could alternatively speculate that this primary differentiation defect could occasionally manifest itself also in the PNH defect The tests relevant to PNH were carried out relatively late in the course of the disease and consequently it is difficult to evaluate which of the two diseases is the primary one In patient 7 there was a history of voiding dark urine six years before signs of myelofibrosis were found This cannot be further evaluated In patient 8 frank haemoglobinuria appeared after the diagnosis of myelofibrosis had been established and the appearance of two erythrocyte populations 18 months after the first chromium 51 autosurvival curve had revealed only one population also points to myelofibrosis as the primary disease None of these facts however prove this sequence Haemoglobinuria may have gone unnoticed for long periods or it may have developed late in the disease even if the PNH defect might have been present for a long period of time Also the appearance of two erythrocyte populations may merely indicate that by that time the PNH cells may have become *sufficiently numerous to be revealed in the auto-*survival curve Clearly more observations of combined PNH and myelofibrosis are needed before their relationship can be defined Finally it should be recognized that although myelofibrosis is the most likely diagnosis in patient 7 the diagnosis is not definitely established The enlarged liver and spleen during at least three years the

elevated leukocyte count with a shift towards immature cells the thrombocytosis with giant platelets the normoblasts in peripheral blood the pronounced anisocytosis and the moderate poikilocytosis are all in favour of the diagnosis of myelofibrosis Arguments although not decisive against the diagnosis were that the bone marrow was readily aspirated erythropoiesis was not present in the spleen and was not ineffective as estimated from Fe 59 studies and serum uric acid levels were normal The balance of the evidence would favour myelofibrosis In patient 8 the diagnosis of myelofibrosis appears well substantiated spleno-hepatomegaly leucocytosis with metamyelocytes increased numbers of basophilic granulocytes slight thrombocytosis before the operation pronounced poikilocytosis with tear forms normoblasts in peripheral blood increased basal metabolic rate and the slight hyperuricaemia are all in favour of the diagnosis The biopsy from the iliac crest with reticulum cells and megakaryocytes as the predominant cells and the spleen with myeloid metaplasia with red white eosinophilic and megakaryocyte like cells are also typical of myelofibrosis only fibrosis could not be demonstrated That fibrosis may be absent in the early stages of myelofibrosis is well known (2)

Among the remaining four patients evidence of a concomitant rare disease was found in patient 4 who developed a positive direct Coombs test and cutaneous manifestations with a histological picture highly suggestive of thrombotic thrombocytopenic purpura (Fig 1) Also patient 7 had a transient positive Coombs test These manifestations will be discussed below In the remaining patients there was no indication of any other blood disease and accordingly their PNH defect must be classified as primary

COMPLICATIONS OF PNH

Competitive causes of anaemia

The anaemia due to the PNH defect per se is haemolytic In addition ineffective erythropoiesis and anatomical marrow hypoplasia are sometimes present To complicate matters even further other mechanisms of anaemia may also be operative Iron deficiency may develop due to urinary iron loss and folic acid deficiency may appear perhaps as a result of the increased demands during haemolysis Occasionally the Coombs test is positive This could suggest that immune factors may play a role but might just as well reflect the fact simply that a high proportion of the red cells are coated with transferrin

Iron deficiency develops as a result of the iron lost in the urine as haemosiderin and haemoglobin The amount of iron lost in PNH has been estimated by a number of authors (reviewed in ref 20) The results vary considerably probably because it is not always stated whether the lost iron is due to haemosiderinuria haemoglobinuria or both. In these reports the estimated iron loss varies between 1 and 16 mg a day In a long term study Dac e (8) found the average iron loss to be 10 mg a day in one of his patients corresponding to an estimated loss of iron of 48 g over a period of 11 years of disease In a detailed study Hartmann (20) found the average urinary loss due to non haemoglobin iron to be 1 8–7 8 mg/day To this chronic loss of iron should be added the appreciable amounts of iron which may be lost during occasional major attacks of haemoglobinuria Thus the development of iron deficiency in PNH patients is readily understandable Nevertheless iron deficiency in PNH is rather seldom reported (6) The probable reason is that the diagnosis of iron deficiency anaemia superimposed on the PNH anaemia may be difficult Frequent checks with respect to iron deficiency during the course of the disease should be made In the careful study by Hartmann et al (20) iron deficiency was found in all seven patients studied

The usual signs of iron deficiency—low serum iron high TIBC low MCHC and low MCV—do not always give clear cut answers in haemolytic anaemia in which the serum iron tends to be high because of high plasma iron turnover and the MCV tends to be high and the MCHC low because of skipped divisions The best method of diagnosing iron deficiency is to evaluate the amounts of stainable iron in the bone marrow Five of our eight patients revealed iron deficiency at one time or another Four of these patients were put on iron medication the results of this treatment will be reported below

A megaloblastic component is rarely found in PNH Few cases have been published in which folic acid deficiency was found (25 31 32) One of our patients (no 6) developed megaloblastic erythropoiesis during the course of his illness at a

Table II *Clinical features*

Pat no	Presentation	Hypercoagulability	Thromboses	Iron deficiency	Abnormal EEG	Effect of prednisone	Coombs test
1	Attacks of abdominal pain	+	Mesenteric (?)	+	Not examined	Not given	–
2	Long standing pancytopenia	–	Cerebral	+	+	–	–
3	Long standing pancytopenia	–	Lower extremity Renal venous	–	Not examined	+	–
4	Long standing pancytopenia TTP (?)	+	Dermal	+	+	+	+
5	Hypoplastic anaemia	+	Mesenteric (?)	+	+	?	–
6	Hypoplastic anaemia	+	–	–	–	+	
7	Myelofibrosis (?)	+	Lower extremity	–	+	?	+
8	Myelofibrosis	+	Lower extremity	?	+	–	–

time when there was haemolysis but the acid haemolysis test was still negative Serum B_1 was normal but serum folic acid was low His diet seemed adequate and malabsorption could not be demonstrated thus no other reason was found for the folic acid deficiency than the haemolysis with its increased demands Folic acid administration was followed by a significant rise in haemoglobin concentration

Coombs positivity An immunological basis for PNH has never been established Yet it appears that a positive direct Coombs test is found more often in PNH patients than could be accounted for if the coincidence were fortuitous (6) Two of our patients had a positive direct Coombs test at one time during their illness (patients 4 and 7) The positive Coombs tests were observed at times when the reticulocyte counts were only about 2 1 and 5 0 % respectively Therefore it is not likely that the Coombs positivity was due to *transferrin coating of young red cells* In one of them (no 7) the positive Coombs test was transient and the significance uncertain In the other patient the positive Coombs test was associated with other evidence of immunological disease presence of the antinuclear factor in the blood and histological features highly suggestive of thrombotic thrombocytopenic purpura (Fig 1) Whereas the diagnosis of TTP seemed certain on histological grounds the lack of leucocytosis and neurological symptoms prompt response of symptoms to prednisone and the subsequent benign clinical course are arguments against TTP To our knowledge a similar histological picture has not been reported in PNH patients

The occurrence of a positive Coombs test in PNH is usually considered to be a transient episode which is associated with an exacerbation of the anaemia (6) This view is supported by the present observations

Hypercoagulability—thrombotic complications

Thrombotic manifestations are often encountered in PNH patients The thromboses may occur everywhere—in the extremities in the brain in the abdomen (6)—the commonest place probably being the mesenteric vessels It seems reasonable to attribute the numerous attacks of abdominal pain experienced by these patients to small mesenteric thromboses an assumption sometimes verified at operation (4)

The problem of why thromboses occur so often in a disease in which thrombocytopenia is a frequent feature has not yet been solved but there seems to be general agreement that somehow the thrombotic complications are related to periods of accentuated haemolysis (19) Several authors have demonstrated a state of hypercoagulability even if no agreement has been reached as to which components are responsible for these changes (15)

Coagulation studies were performed in seven

of our patients in three cases tests were made both at the time of moderate haemolysis and during haemolytic attacks with haemoglobinuria All but one of the seven patients examined had a state of hypercoagulability and the three patients examined during haemolytic attacks displayed increased hypercoagulability during attacks as compared to the more quiescent periods Indications of thromboses were found in seven patients (see Table II) The detailed results of coagulation studies will be reported separately (1)

EEG changes

Six of our patients had electroencephalograms (EEG) recorded Varying degrees of abnormality were found in five patients (nos 2 4 5 7 and 8) Four of these patients had spikes universally or in the temporal leads One patient displayed a rather low dominant frequency Three patients were examined during sleep One of these had no changes from the state when she was awake (no 5) whereas the other two (nos 2 and 8) had accentuated abnormalities during sleep

The significance of these findings is at present completely unresolved Three possibilities should be evaluated the changes may be purely incidental the EEG changes may be secondary to the PNH defect or the changes may be indications of a cerebral lesion aetiologically related to the PNH erythrocyte defect

Even if the finding of five abnormal EEGs in six patients is greater than could be expected on a chance basis the relatively small number of patients precludes conclusions concerning the specificity of the changes Moreover alternative explanations of the changes are conceivable in some of the patients patient 4 may have suffered from thrombotic thrombocytopenic purpura patient 5 had a history of brain concussion and a hemicrania like headache and patient 7 had a malignant tumour which might have metastasized to the brain even if no clinical symptoms supported this assumption In the remaining two patients there was no other explanation of the EEG abnormality

That the EEG changes may be secondary to the PNH defect appears to be a definite possibility in view of the fact that thrombotic manifestations often occur in the cerebral vessels One of our patients had a history of a cerebral thrombosis at the age of 55 It is well possible that in the remaining patients thromboses may have passed unnoticed clinically and merely manifested themselves in abnormal EEGs

The third possibility that the EEG changes may be indicative of a primary brain lesion operative in the pathogenesis of PNH offers ample material for speculations However no matter how intriguing such guesswork may be speculations on the possible significance of the brain and of possible defects in the brain in PNH remain at present futile From the present data it appears advisable to perform systematic EEG studies in all cases of PNH

TREATMENT OF PNH

Even if no specific treatment for the PNH defect exists some aspects of the disease are amenable to therapy Treatment must be adjusted to the particular problems of the individual patient Since the problems may vary from time to time the indications for therapy must constantly be reviewed

In acute haemolytic crises *transfusions with washed erythrocytes* may be life saving These crises may be life threatening because of the anaemia and—in rare cases—because of acute renal failure due to haemoglobinuria Renal impairment was seen in patient 8 who developed haemoglobinuria and anuria probably due to a concentrated plasma protein infusion

Transfusions with whole blood are extremely dangerous in PNH probably because the transfused blood provides complement Transfusions to PNH patients must therefore be given as washed erythrocytes Such transfusions are effective in two ways they restore the haemoglobin concentration and they tend to diminish the production of defective erythrocytes in the marrow (10) This latter effect was demonstrated in patient 7 When he was transfused with washed erythrocytes the haemoglobin concentration rose from 7 5 g/100 ml to 11 2 g/100 ml Concomitantly the reticulocytes fall from around 500 000/μl to 90 000/μl

Transfusions are indicated in severe haemolytic crisis and may also be indicated if during the course of the disease an acceptable haemoglobin level cannot be maintained in other ways During recent years there has been a tendency to treat even slight anaemia with multiple transfu

sions This is hardly justifiable in view of the dangers of multiple transfusions including trans fusion haemosiderosis which exists also in PNH in spite of the chronic urinary iron loss Whereas no stainable iron was found in extrarenal tissues in early reports, the past 10–15 years have brought many reports of stainable iron in extra renal tissues in multiple transfused cases (20)

As previously mentioned iron deficiency de veloped in five of our cases Four of these pa tients were put on *treatment with oral iron* during the time of study One of these (no 8) was operated shortly after therapy had been initiated and the post-operational period precludes assess ment of the value of this treatment Also patient 4 was difficult to evaluate because prednisone was given at the same time In patients 1 and 5 the value of the iron treatment can hardly be disputed in both of them the haemoglobin con centration increased by 3–4 g/100 ml over a relatively short period of time It has been re ported that iron medication may provoke haemo lytic episodes In a thoroughly examined group of patients gross haemoglobinuria developed in many of the patients on iron therapy (20) It is difficult to state whether or not the iron therapy has provoked an increased number of haemolytic episodes in our patients Patient 1 may have ex perienced more haemolytic attacks while on oral iron but patient 4 has never experienced gross haemoglobinuria and there is nothing to suggest more frequent haemolysis in patient 5

The megaloblastic anaemia seen in patient 6 at one time during his illness was successfully treated with *folic acid* In spite of its rarity the possibility of secondary folic acid deficiency should always be kept in mind

Treatment with prednisone is usually con sidered to be of no value in PNH and has even been warned against as thrombotic complica tions have been attributed to this treatment (10) Nevertheless there are some claims of beneficial results with prednisone therapy (15) In the present series prednisone was given to seven of our eight patients In two patients (nos 2 and 8) no effect was seen and in one patient (no 7) the effect was equivocal In the remaining four patients however a prednisone effect was seen at one time or another during the course of the illness In two patients pred isone was effective during the initial period of aplastic anaemia com bined with haemolysis In one of these patients prednisone had no effect when the PNH defect was fully developed (no 5) The other patient (no 6) is at present in full haematological remis sion on a combined treatment of prednisone anabolic steroid and folic acid Patient 3 en joyed an almost normal haemoglobin concentra tion during one year of prednisone treatment after at least 15 years of pronounced anaemia During the same period Ham s test turned nega tive an event which is extremely rare In the last patient (no 4) with a positive Coombs test and histological features of thrombotic thrombo cytopenic purpura the effect of prednisone could have been on the possible immunological com ponent in the haemolytic anaemia However in this patient the haemoglobin concentration rose to a level not seen during the past ten years of her illness during which time no evidence of im munological disease had been found Still the effect of prednisone cannot be evaluated because at the same time iron was given

In our opinion the possible effect of prednisone *in PNH patients cannot be predicted A trial of* prednisone should be made in all PNH patients when anaemia which cannot be explained from iron or folic acid deficiency is present

A beneficial effect of *anabolic steroids* in PNH has been reported (20) This treatment was given to four of our patients In patient 2 the treatment has just been initiated and patient 5 only received this treatment for a relatively short period of time four years before evidence of the PNH defect was found In patient 8 no effect of the treatment was seen during a six months trial As previously mentioned, patient 6 is at present in full remission on a combined treatment of pred nisone anabolic steroid and folic acid

Thus the effect of prednisone and anabolic steroids in the treatment of PNH seems varying and unpredictable This may simply be due to the capricious course of PNH with incidental improvements which erroneously may be attrib uted to the treatment given If one prefers to accept that prednisone and anabolic steroids sometimes do have a therapeutic effect in patients with the PNH defect the unpredictability might be explained in terms of the drug effect on an underlying condition PNH erythrocytes are be lieved to develop in several states in which nor mal erythropoiesis is impaired or haemolysis

exists If the primary disorder is corrected by corticoid or anabolic steroid treatment anaemia is relieved this might secondarily result in suppression of the abnormal PNH red cell clone with diminished PNH red cell production haemolysis would also decrease

The use of *anticoagulant therapy* against haemolysis and thrombotic complications has been advocated (6 10) Heparin and dicoumarol have both been used The reports concerning heparin are conflicting (24) Crosby reports in vitro trials showing that in small doses heparin increases haemolysis whereas in large doses haemolysis is diminished This could not be confirmed in an other study (16) In vivo trials have been associated with both increased and decreased haemolysis (16 27) Some are of the opinion that heparin is too dangerous to be used in treatment of PNH (6) Dicoumarol has been advocated in the treatment of PNH both as an inhibitor of haemolysis and for the prevention of thrombotic episodes (6) Whereas the former effect of dicoumarol is not universally accepted most authors agree that dicoumarol has a place in the treatment of PNH patients with thrombotic complications (6 10)

Our experience with dicoumarol treatment is limited to one patient (no 1) who was put on permanent dicoumarol treatment because of recurrent episodes of abdominal pain During eight months of treatment however the attacks of pain have been unaffected

ADDITION IN PROOF

Patient no 8

The patient recovered from the postoperative complications, and the anaemia was alleviated on treatment with testosterone Since the alleviation of the anaemia all signs of the PNH defect have disappeared (negative Ham s test, negative sucrose haemolysis test, no haemoglobinuria normal ACHE activity in the red cells) and a new chromium 51 autosurvi al showed evidence of only one erythrocyte population with a half life of 24 days

REFERENCES

1 Amris C J & Hansen, N E Acta med scand 184 551 1968
2 Andreasen, A. P Myelofibros s Munksgaard Copenhagen 1958
3 Auditore J V Hartmann, R. C Flexner J M & Balchum O J Arch Path. 69 534 1960
4 Blum, S F & Gardner F H New Engl J Med 74 1137 1966
5 Brus, I & Lewis, S M Brit J Haemat 5 348 1959
6 Crosby W H Blood 8 769 1953
7 Crosby W H & Benjamin, N R. Blood 15 505 1960
8 Dacie J V & Lewis S M Brit J Haemat 7 44? 1961
9 Dacie J V Proc roy Soc Med 56 587 1963
10 — The haemolytic anaemias IV Churchill London 1967
11 Dameshek W & Fudenberg, H Arch intern Med 99 20? 1957
12 De Sandre G Ghiotto G & Mastella G Acta med patav 16 310 1956
13 Emerson P H & Wilkinson, J H Brit J Haemat 12 678 1966
14 Firkin, B J Beal R. W & Mitchell G Aust Ann Med 12 26 1963
15 Firkin B J & Wiley J S Progr Hemat 5 45 1966
16 Fritsche W & Martin H Klin Wschr 35 1166 1957
17 Hansen N E & Nielsen J B In preparation.
18 Hansen N E Acta med scand 184 543 1968
19 Hartmann, R. C & Jenkins D E Jr Blood 25 850 1965
20 Hartmann R. C Jenkins D E Jr McKee L C & Heyssel, R. M Medicine (Baltimore) 45 331 1966
21 Hartmann R. C & Jenkins, D E Jr New Engl J Med 275 155 1966
22. van der Helm, R. C Clin. chim. Acta 7 124 1962
23 Herz, F Kaplan, E & Stevenson, J H Jr Nature (London) 200 901 1963
24 Hinz, C F Progr Hemat. 5 60 1966
25 Hirsch J Ungar B & Robinson, J S Aust Ann Med 13 24 1964
26 Jørgensen, K. Scand J clin. Lab Invest 11 282, 1959
27 Lasch, H G Linke A & Sessner H H Acta haemat (Basel) 13 366 1955
28 Laursen T Scand J clin Lab Invest 11 134 1959
29 Lewis, S M & Dacie J V Brit J Haemat. 13 236 1967
30 Metz, J Bradlow B A Lewis S M & Dacie J V Brit J Haemat 6 372 1960
31 Metz, J Stevens K., van Rensburg, N J & Hart D Brit J Haemat 7 458 1961
32 Pavlic G J & Bouroncle B A New Engl J Med 273 789 1965
33 Rosse W F & Dacie J V J clin. Invest 45 736 1966
34 Wieme R. J Agar gel electrophoresis Elsevier Amsterdam 1965

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THE SUCROSE HAEMOLYSIS TEST IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

Studies on Erythrocytes and Bone Marrow Cells

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Abstract The sucrose haemolysis test has been carried out in seven PNH patients twenty normal persons and fifty patients with haemolysis not attributable to PNH It was concluded that the test is valuable in the diagnosis of PNH since it appears to be specific for this disease seems to be more sensitive than the acidified serum (Ham s) test and is quite easy to perform The significance of slightly positive reactions in three patients with haemolysis other than PNH is discussed The more simplified test the sugar water test has been found to be unreliable The sucrose haemolysis test has also been carried out on nucleated cells from the bone marrow of seven patients with the PNH defect These cells were not susceptible to lysis by the sucrose solution

The diagnosis of paroxysmal nocturnal haemoglobinuria (PNH) is based mainly on the demonstration of intravascular haemolysis decreased activity of acetylcholine esterase in the red cells and—considered a sine qua non—a positive Ham s test (the acidified serum test)

Until recently it was generally accepted that PNH was a disease of its own and that a positive Ham s test was diagnostic of this disease Evidence of PNH developing in the course of aplastic anaemia (3) and myelofibrosis (5) has however called for a reconsideration of the concept of PNH In this period of reevaluation it is desirable to establish the broadest possible basis for the diagnosis and therefore additional diagnostic criteria are of value

Recently new diagnostic tests for the PNH defect have been developed viz. the complement lysis test (15) and the sucrose haemolysis test (8) Experience with these tests is still very limited The purpose of the present work was to study the usefulness of the sucrose haemolysis test performed with peripheral red cells in the diagnosis of PNH In addition the sucrose haemolysis test was performed on nucleated bone marrow cells

METHODS

The sucrose haemolysis test on peripheral red cells

A 10 sucrose solution was prepared by dissolving 10 g of sucrose in 100 ml of distilled water The pH of this solution is approximately 6 3 Buffering was not used 0 85 ml of a freshly prepared sucrose solution was mixed with 0 05 ml of autologous serum and 0 1 ml of a 50% suspension of washed erythrocytes from oxalated blood After incubation for 30 min at 37 C the mixture was centrifuged and the supernatant observed for visible haemolysis. If haemolysis was present the test was repeated with homologous compatible serum instead of autologous serum

The sugar water test

One ml of oxalated blood was mixed with 9 ml of a freshly prepared sucrose solution made by dissolving 10 g of sucrose in 100 ml of distilled water The mixture was incubated for 30 min at 37 C centrifuged and the supernatant observed for visible haemolysis

The sucrose haemolysis test on marrow nucleated cells

Marrow was aspirated from the sternum collected in oxalate transferred to capillary tubes (internal diameter 1 5 mm) and centrifuged at 3000 g for 20 min In this way the marrow aspirate was separated into four sharply demarcated layers 1 fatty layer 2 marrow plasma, 3 buffy coat, containing the nucleated cells and 4 erythrocytes The sucrose haemolysis test was carried out on the nucleated cells using the same volumes of sugar cells and serum as indicated for the sucrose haemolysis test on peripheral red cells After centrifugation at 3000 g for 30 min the activity of lactate dehydrogenase (LDH) in the supernatant was determined as an indicator

of lysis The activity was compared with the activity in a control tube containing isotonic sodium chloride instead of the sucrose solution

Lactate dehydrogenase was determined by Laursen s method (12)

Acetylcholine esterase activity in the red cells was measured as indicated by Jorgensen (11) the normal range being 16 0–23 2 μmol/min/g haemoglobin

MATERIAL

Seven patients with the PNH defect were studied The histories and the diagnostic criteria of these patients are reported elsewhere (5) In these patients the sugar water test was carried out on erythrocytes and the sucrose haemolysis test on erythrocytes and marrow nucleated cells

The sucrose haemolysis test was also carried out in 20 apparently healthy persons and in 50 patients with various diseases associated with haemolysis The criteria for the presence of haemolysis were constant or decreasing haemoglobin concentration associated with increased reticulocyte counts with no evidence of bleeding or red cell mass regeneration. The following diseases with haemolysis were examined (the number of patients in each group is shown after the diagnosis) myelofibrosis 9 autoimmune haemolytic anaemia 4 acquired idiopathic haemolytic anaemia 10 microangiopathic haemolytic anaemia 1 idiopathic pancytopenia 4 aplastic anaemia 2 systemic lupus erythematosus 1 Hodgkin s disease 2 multiple myeloma 1 Waldenstrom s macroglobulinaemia 1 acute myeloblastic leukaemia 3 chronic myelogenous leukaemia 2 acute lymphoblastic leukaemia 2 and chronic lymphatic leukaemia 8

RESULTS

The sucrose haemolysis test on peripheral red cells was positive in all seven patients with PNH irrespective of whether autologous or homologous compatible serum was used

The sugar water test was positive in only three of the seven patients with the PNH defect Moreover this test was not consistently positive in these three patients

The 20 normal persons all had negative sucrose haemolysis tests Of the 50 patients with haemolytic disorders other than PNH 47 had negative sucrose haemolysis tests Three patients had weakly positive tests

The first patient was a 61 year old woman with a severe autoimmune haemolytic anaemia Haemoglobin was 3 9 g/100 ml reticulocytes 63% serum bilirubin 5 6 mg/100 ml plasma haemoglobin 29 mg/100 ml and serum LDH 90 units (normal upper limit 23 units) with an isoenzyme pattern compatible with haemolysis In the serum an agglutinating erythrocyte antibody could be demonstrated (titre by Coombs technique 2048 at 37 C) Haemoglobinuria was present Ham s and Crosby s tests were negative the acetylcholine esterase activity in the erythrocytes was normal (17 5 units) The sucrose haemolysis test was weakly positive in autologous serum but negative in homologous compatible serum

The *second patient* was a *61 year-old* man with myelofibrosis Haemoglobin was 6 4 g/100 ml reticulocytes 2 5% serum bilirubin 1 9 mg/100 ml and LDH 33 units with an isoenzyme pattern compatible with haemolysis Coombs direct test was negative Neither haemoglobin nor haemosiderin was present in the urine Ham s and Crosby s tests were negative The acetylcholine esterase activity in the erythrocytes was low (14 0 units) The sucrose haemolysis test was weakly positive in autologous and homologous compatible serum

The third patient was a 46 year old woman with myelofibrosis Haemoglobin was 8 9 g/100 ml reticulocytes 1 4–2% and serum bilirubin 1 4 mg/100 ml An autosurvival study with chromium 51 tagged erythrocytes showed a half life of 15 days with no evidence of a double population Serum LDH was normal Coombs direct test was negative There was no haemoglobin or haemosiderin in the urine Ham s and Crosby s tests were negative The acetylcholine esterase activity in the erythrocytes was low (15 5 units) The sucrose haemolysis test was weakly positive in autologous and homologous compatible serum

The sucrose haemolysis test was performed on *nucleated bone marrow cells* from the seven patients with PNH No increase in the supernatant LDH activity was found in any case when comparing the sucrose and the saline medium

DISCUSSION

The theoretical background for the sucrose haemolysis test is not yet fully established However it has been shown that solutions of low ionic strength such as the sucrose solution used in this test is able to induce complement coating of red cells (13) and it has been demonstrated that the PNH erythrocyte is sensitive to lysis by abnormally small amounts of complement (15)

The initial report on this test (8) demonstrated that it was positive in eight PNH patients whereas it was negative in 25 normal persons in 50 patients with various non haematological disorders and in 75 patients with various haematological diseases These early results have later been expanded so that at present the test has been performed on more than 200 patients (10) the test still appearing to be specific for PNH

The present work supports these reports on the value of the sucrose haemolysis test. The test was positive in all the seven PNH patients examined and it was clearly negative in the 20 normal persons and in 47 of the 50 patients with haemolysis which could not be attributed to PNH The positive outcome of the test in the three patients in whom the diagnosis of PNH could not be definitely established calls for some comments

In the first of these patients the test was positive in autologous serum but negative in homologous compatible serum In the initial report (8) it was stated that the test might be positive in acquired immune haemolytic anaemias due to the presence of haemolysins even if this had not been observed Our patient illustrates this point The patient had a strong erythrocyte antibody in her serum and whereas the test was positive in autologous serum it was negative when homologous serum was used

The two patients with myelofibrosis had weakly positive tests both in autologous and homologous serum In both patients Coombs direct test was negative Ham s and Crosby s tests were negative and thus the diagnosis of the PNH defect could not be established Both patients however had low acetylcholine esterase activity in their red cells and it is thus possible that these patients did have the PNH defect to a mild degree if this interpretation is correct it could mean that the sucrose haemolysis test is a more sensitive indicator of the PNH defect than the classical tests This assumption is supported by the findings in one of our patients with an established diagnosis of PNH At a time when this patient had been intensively transfused and consequently most of his red cells were donor erythrocytes Ham s and Crosby s tests became negative whereas the sucrose haemolysis test remained weakly positive

Our experience with the more simplified version of the test *the sugar water test* was not favourable Only three of the seven PNH patients examined had positive tests and the test was not consistently positive in these three patients The unfavourable experience with this simple test is in contrast with the results of the initial report (9) in which the test was positive in all of the eight PNH patients examined

The simple technique of the sucrose haemolysis test makes it applicable in studies of possible lysis of cells other than erythrocytes In the present work the sucrose haemolysis test was carried out on nucleated bone marrow cells Even if it is known that PNH haemolysis in vivo is due mainly to intravascular destruction the possible contributory role of intramedullary haemolysis (= ineffective erythropoiesis) has not been fully elucidated Crosby is of the opinion—based on studies of haemoglobin formation—that PNH is similar to pernicious anaemia in this respect i e that ineffective erythropoiesis is prominent in the haemolytic mechanism (2) Radioisotope studies using Fe 59 have yielded contradictory results although most investigators agree that erythropoiesis in PNH is effective (1 4 9) Other studies using lactate dehydrogenase liberated into marrow plasma as an indicator of lysis also suggest that intramedullary haemolysis is not prominent in PNH (5 6)

Information on the susceptibility of marrow nucleated cells to in vitro haemolysis is scarce Crosby (2) and Nussey et al (14) applied the acidified serum test (Ham s test) to bone marrow cells and reported that marrow nucleated cells were destroyed by this procedure In the present work the sucrose haemolysis test was performed on marrow nucleated cells and liberated LDH used as an indicator of lysis The use of this enzyme for this purpose seems justified LDH is known to exist in bone marrow nucleated cells and determination of the enzyme activity can be used as an indicator of intramedullary haemolysis in pernicious anaemia (16) Furthermore when the sucrose haemolysis test is performed on peripheral erythrocytes LDH is liberated together with haemoglobin (7)

Liberation of LDH from the nucleated marrow cells exposed to the sucrose haemolysis system could not be demonstrated in any of the seven PNH patients studied This may be considered an additional argument against the postulated role

of ineffective haemopoiesis in PNH Thus there is increasing evidence that erythropoiesis is not ineffective in PNH If this is accepted one is faced with the problem why PNH reticulocytes are susceptible to lysis whereas the only slightly younger nucleated red cell precursors are not An obvious difference between the two types of cells is the presence of the nucleus and of RNA synthesis in the nucleated cells However whether or how this could confer any protection on the cells must remain a matter of speculation

REFERENCES

1 Beal R. W., Kronenberg, H & Firkin B G Amer J Med 37 899 1964
2 Crosby W H Blood 8 769 1953
3 Dacie J V & Lewis S M Brit J Haemat 6 362 1961
4 Flatmark T & Myhre E Acta med scand 173 53 1963
5 Hansen, N E & Killmann S Aa Acta med scand 184 525 1968
6 Hansen, N E & Andersen, V Acta med. scand 183 581 1968
7 Hansen, N E Unpublished data
8 Hartmann R. C & Jenkins D E Jr New Engl J Med 275 155 1966
9 Hartmann R C., Jenkins D E., Jr McKee L C & Heyssel R. M Medicine (Baltimore) 45 331 1966
10 Jenkins D E., Jr Hartmann, R C & Kerns A. L J clin Invest 46 753 1967
11 Jørgensen K Scand J clin. Lab Invest 11 282 1959
12 Laursen, T Scand J clin Lab Invest 11 134 1959
13 Mollison P L & Polley M J Nature (London) 203 535 1964
14 Nussey A M & Dawson, D W Blood 11 757 1956
15 Rosse W F & Dacie J V J clin Invest 45 736 1966
16 Yakulis V J Gibson C W & Heller P Amer J clin Path 38 378 1962

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SLEEP RELATED PLASMA HAEMOGLOBIN LEVELS IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

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Abstract Plasma haemoglobin concentrations have been determined in seven patients with the PNH defect over 24-hour periods, and in four PNH patients during short periods of sleep in daytime It has been demonstrated that although a rise in plasma haemoglobin during sleep is frequent it is not a constant finding Contrary to previous reports a splenectomized patient showed characteristic sleep-related rise in plasma haemoglobin during sleep The observations confirm that plasma haemoglobin levels in PNH patients often vary inversely with the natural variations in plasma cortisol concentrations.

As first demonstrated in the careful studies by Ham (4) the regular periods of increased haemolysis seen in patients with paroxysmal nocturnal haemoglobinuria (PNH) are related to sleep rather than to the night as implied in the name of the disease Ham studied the diurnal variations in plasma haemoglobin over several days and nights and found increased concentrations of plasma haemoglobin during sleep irrespective of whether the patients slept at night or during the day

Surprisingly few similar studies have been carried out and at present it is not known whether or not the sleep-associated hyperhaemolysis is a constant finding in PNH patients (2)

The present work was carried out in order to examine the constancy of sleep-related hyperhaemolysis in patients with the PNH defect it also provided an opportunity to study the effect of splenectomy and corticosteroid medication on PNH hyperhaemolysis

MATERIAL AND METHODS

Seven patients with the PNH defect were studied The histories of these patients are reported elsewhere (5) (patients 1 2, 4 5 6 7 and 8 of that study)

Plasma haemoglobin concentrations were determined every four hours over a 24-hour period Furthermore plasma haemoglobin concentrations were determined in four patients during short periods of sleep in daytime

Immediately after venipuncture the oxalated blood was centrifuged at 3000 g for 15 min Plasma haemoglobin was measured as described by Harboe (6)

RESULTS

The results are shown in Figs 1 and 2 All patients but two showed increased levels of plasma haemoglobin during sleep at night the maximum level being reached at 12 p m or 4 a m One patient (no 6) did not show any significant diurnal variations and one patient (no 4) had the lowest plasma haemoglobin levels when asleep at night

In all the four patients examined the plasma haemoglobin level rose markedly during short periods of sleep in daytime with an approximately twofold increase in plasma haemoglobin As seen in Fig 2 the increased level of plasma haemoglobin during sleep quickly fell to the pre-sleep level when the patient woke up

DISCUSSION

This study thus demonstrates that increased levels of plasma haemoglobin during sleep are frequently but not invariably found in patients with the PNH defect Also the study demonstrates that the changes in plasma haemoglobin concentrations associated with sleep are quickly initiated and terminated

The two patients who did not show increased levels of plasma haemoglobin during sleep merit further comments Patient 6 did not show any

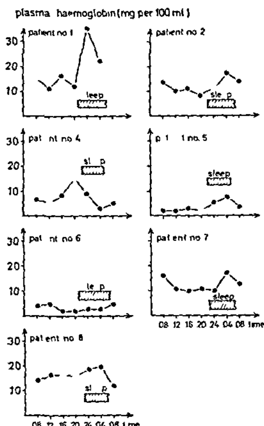


Fig 1 Diurnal variations in plasma haemoglobin

significant diurnal variations which might be explained by the fact that this patient only had a very mild PNH defect Patient 4 had marked diurnal variations the highest plasma haemoglobin values being reached when she was awake and ambulatory and the lowest values when she was asleep at night thus presenting a haemolytic pattern contrary to the pattern usually accepted for PNH haemolysis Four months prior to this study this patient developed multiple thromboses in the dermal vessels and it must be considered whether part of the haemolysis seen in this patient could be attributed to the mechanism operative in microangiopathic haemolytic anaemia In this type of haemolytic anaemia haemolysis is strongest when the cardiac output is the greatest (8 11) which might explain why the plasma haemoglobin concentration was greatest when the patient was awake and ambulatory However at the time of the present study the dermal thromboses had vanished and at no time could any bizarre red cells be demonstrated in blood smears a feature considered a prerequisite for the diagnosis of microangiopathic haemolytic anaemia (1) At present then the only known haemolytic disorder in this patient is PNH which may suggest that even if sleep is usually associated with increased levels of plasma haemoglobin in patients with PNH this pattern of increased haemolysis is not a constant finding

The mechanism for the sleep associated hyper haemolysis in PNH patients is not known The in vitro test for PNH the acidified serum test (Ham s test) is based on the fact that PNH erythrocytes haemolyse when exposed to acidified serum and naturally a similar mechanism has been suggested for the in vivo haemolysis It has been suggested that the CO retention seen during sleep migh lower the pH in the blood and consequently induce haemolysis However a lowering of pH to such a degree as to induce haemolysis has not been demonstrated and it has been shown that hype haemolysis is still seen during sleep even when pCO is kept artificially low by having a patient sleep in a respirator

Ham was of the opinion that even if no significant lowering of pH in the blood could be demonstrated the local circulation in the spleen might become sufficiently sluggish during sleep to lead to a significantly low pH and thus induce haemolysis (3) The basis for this assumption was that in one patient the previously found increase of plasma haemoglobin during sleep could no longer be demonstrated after splenectomy This observation has also been made by others (7) In the present study patient 5 had been splenectomized four years previously but still sleep-related

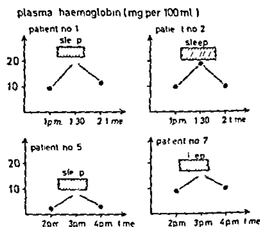


Fig 2 Variations in plasma haemoglobin during short periods of sleep in daytime

hyperhaemolysis was clearly seen as demonstrated in the accompanying figures It thus appears that the spleen is not alone responsible for the hyperhaemolysis seen during sleep

Recently it has been noted that the diurnal changes in plasma haemoglobin in PNH patients vary inversely with the diurnal changes in plasma cortisol and it has been suggested that these low plasma cortisol concentrations might have some bearing on the increased haemolysis seen during sleep at night (9) Based on this assumption Morley et al (10) gave glucocorticoids to a patient with PNH and claimed that haemolysis at night was diminished

The present work supports the observation that the highest levels of plasma haemoglobin are *most frequently seen* when plasma cortisol is normally lowest Whether or not the very fast changes in plasma haemoglobin seen *during the* short periods of sleep in this study can be correlated to low plasma cortisol levels *is not known*

In the present study patients 4 5 6 and 8 were on prednisone treatment at the time of study The doses of prednisone given were as follows patients 4 and 6 5 mg three times a day patient 5 10 mg four times a day and patient 8 10 mg three times a day In the accompanying figures it is seen that this treatment did not significantly suppress the sleep related hyperhaemolysis

REFERENCES

1 Brain, M C. Dacie J V & Hourihane D O B Brit J Haemat 8 358 1962
2 Dacie J V The haemolytic anaemias IV Churchill London 1967
3 Ham, T H New Engl J Med 217 915 1937
4 — Arch intern Med 64 1271 1939
5 Hansen N E & Killmann S Aa Acta med scand 184 525 1968
6 Harboe M Scand J clin Lab Invest. 11 66 1959
7 McIlvaine S K. & Beard M F Blood 6 936 1951
8 Miller D S Mengel C E *Kremer W B.*, *Gut*terman J & Senningen, R. Ann intern. Med 65 210 1966
9 Morley A. A Brit J Haemat 13 310 1967
10 Morley A A., Baker L R J Beardwell C. G & Burke C W Lancet 7530 448 1967
11 Sears D A & Crosby W H Amer J Med., 39 341 1965

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COAGULATION AND FIBRINOLYTIC STUDIES IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

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Abstract Seven patients with paroxysmal nocturnal haemoglobinuria (PNH) have been examined during quiescent haemolysis as well as during haemolytic exacerbations with thrombotic manifestations In six of the patients hypercoagulability was observed as indicated by short kaolin-cephalin time accelerated thromboplastin generation screening test and/or increased activity of factors II+VII and VIII In three cases it was demonstrated that the patient plasma could accelerate the thromboplastin generation of normal plasma No abnormalities were observed in fibrinolysis nor changes indicative of consumption coagulopathy The significance of the demonstrated hypercoagulability is unknown The conclusion of our findings must therefore be limited to the observation that in PNH patients a certain "laboratory hypercoagulable state" can be demonstrated and that this "hypercoagulability" increases during the haemolytic crises

Venous thromboses are a major clinical problem in paroxysmal nocturnal haemoglobinuria (PNH) and the coagulation mechanism in PNH patients has been subjected to a series of investigations The reports on coagulation have been somewhat conflicting in detail However most authors find one or more changes in coagulation indicating a certain activation and hypercoagulability of the clotting mechanism in PNH patients In this report we present some coagulation and fibrinolytic studies in seven PNH patients

MATERIAL AND METHODS

Patients

Case reports on the seven patients of this study are presented elsewhere (10) Coagulation studies were performed on the days listed in Tables I III All the patients were haemolysing at the time of study most of them were examined in a period of quiescent haemolysis as well as during haemolytic exacerbations with thrombotic manifestations

Patient no 1

The first study was carried out during quiescent haemolysis The next study was performed when the patient woke up at night with abdominal pain (abdominal pain in PNH is usually attributed to small mesenteric thromboses) and signs of increased haemolysis with haemoglobinuria The next two studies were carried out on the two subsequent days when the increased haemolysis was subsiding

Patient no 2

Two coagulation studies were performed both during quiescent haemolysis Prior to these studies the patient had experienced a cerebral episode probably due to thrombosis but at the time of study there were no thrombotic manifestations

Patient no 4

The first study was carried out during increased haemolysis at a time when the patient had multiple dermal thromboses. The next two studies were performed during quiescent haemolysis when there were no signs of thrombosis

Patient no 5

The first study was carried out during quiescent haemolysis The next study was performed at a time when the patient had haemoglobinuria and experienced abdominal pain.

Patient no 6

Only one study was made at a time of quiescent haemolysis and no thrombotic manifestations

Patient no 7

Only one coagulation study was carried out at a time when the patient had pronounced haemolysis and thrombosis in a leg vein.

Patient no 8

Several studies were performed The first was made during quiescent haemolysis and at a time when there was

Table I *Coagulation tests*

Test	Patient no 1				Patient no 2		Patient no 4		
	2? 9 66	8 10 66	9 10 66	12 10 66	3 ? 67	3 5 67	7 6 66	7 7 66	30 11 66
Clotting time min } Glass	8½	—	—	4½	8½	7½	5½	7½	8½
Clotting time min } Plastic	23	—	—	11½	21½	18½	1?	15½	15½
Recalcification time sec	170	—	—	160	190	190	210	170	170
Kaolin-cephalin time sec	41 5/ 49 5	45/ 54	40/ 54	39/ 54	46 5/ 48	43/ 47	29/ 47 5	32/ 48	35/ 53
Thromboplastin time sec	14/ 15	17 5/ 17 5	17 5/ 17 5	16/ 16	15 5/ 15 5	13 5/ 14 5	17/ 17 5	16/ 17	16 5/ 18
Factors II + VII	125	85	100	113	100	119	155	130	160
Factor V	—	116	149	150	—	—	220	—	—
Factor VIII	—	138	196	154	—	—	235	—	—
Factor IX	—	—	—	—	—	—	—	—	—
Thromboplastin generation screening test	Accelerated	Accelerated	Accelerated	Slightly accel	Normal	Normal	Accelerated	Accelerated	Accelerated
Thrombin generation test	Normal	—	—	Normal	Normal	Normal	Normal	Normal	Normal
Acceleration of normal thromboplastin generation by patient plasma	—	+ +	+	0	—	—	0	—	+

0 = no acceleration — — + + = acceleration — = not investigated

no sign of thrombosis The following studies were carried out when the patient had undergone splenectomy Because of heavy thrombocytosis the patient was on anticoagulation treatment as indicated below The patient developed deep thromboses in both legs

Reagents

Trisodium citrate 2 H O 3 80 and 3 13

Di potassium EDTA Solution of 2 5 mg/100 ml pH 7.40 dried up in the collecting tubes in amounts equalling 0 1 ml ml of blood

Calcium chloride 1 20 and 1/40 M in distilled water

Owren s buffer 11 75 g of sodium diethylbarbiturate + 14 07 g NaCl in 1570 ml of dist water + 430 ml of 0 1 N HCl pH 7 4 ionic strength 0 15

Phosphate buffers In isotonic saline pH 7 6 and pH 6 1

Thrombin Topostasine® (Roche) dissolved in equal parts of Owren s buffer and glycerol. The stock solution of 100 N I H units/ml was stored at 21 C and diluted with Owren s buffer just before use The dilution was kept at 4 C and discarded after 4–5 hours

Protamin sulphate Solution containing 10 mg/ml in water (Leo Pharmaceuticals Denmark)

Thromboplastin Saline extract of human brain prepared for use in the *p – p* test (22) Stored at 21 C in small aliquots After thawing it was kept at 4 C and discarded after 4–5 hours

Cephalin Human brain cephalin (13) used in optimal concentration diluted with Owren s buffer

Kaolin Kaolin powder washed in distilled water and

Table II *Fibrinolytic tests*

Test	Patient no 1				Patient no 2		Patient no 4		
	22 9 66	8 10 66	9 10 66	12 10 66	3 2 67	3 5 67	7 6 66	7 7 66	30 11 66
Fibrinogen g/100 ml	0 3	0 36	0 44	0 38	0 29	0 27	0 30	0 26	0 ?7
Plasma thrombin time sec	11 11	12 5/ 11	10 5/ 11	10/ 10	10 5/ 10	11/ 10 5	12/ 11	10/ 10 5	11 5/ 10 5
Plasma euglobulin clot lysis time min	> 300	—	—	120	170	190	150	> 240	> 300
Fibrin plates mm[a] } Plasma	—	—	—	—	—	—	9	—	0
Fibrin plates mm[a] } Euglobulin	—	—	—	—	—	—	90	—	50
Plasminogen	—	—	—	—	—	—	—	—	1 2

[a] Values of plasma thrombin time in brackets indicate the protamine thrombin time

Patient no 5		Patient no 6	Patient no 7	Patient no 8						Normal range or simultaneous control
11 11 66	10 1 67	16 1 67	18 11 66	1 2 67	22 3 67	25 3 67	12 4 67	20 4 67	11 5 67	
8½	—	6½	7½	5¾	8½	36	5¼	13¾	8½	6– 12
22½	—	19½	20½	24½	28½	—	27½	26½	37½	15– 25
190	—	170	225	170	170	200	150	—	145	165–220
34/	7/	40/	49 5/	34/	39/	102/	42/	49/	38/	Patient/
48 5	46	47 5	50 5	49	49	54	52	50	45	normal control
17/	19/	19/	19/	17 5/	24 5/	37 5/	18/	22/	17/	Patient/
17 5	20 5	19	17	16 5	15 5	16	15 5	15	16	normal control
173	123	175	80	100	37	33	76	29	100	80–120
—	145	199	88	—	—	—	—	—	—	80–170
—	263	200	293	—	—	—	—	—	—	60–160
—	—	—	15	—	—	—	—	—	—	60–160
Accelerated	Accelerated	Slightly accelerated	Normal	Accelerated	Slightly accelerated	T_m prolonged	Normal	—	Normal	See text
Accelerated	—	Normal	T_m decreased	Slightly accelerated	Slightly accel T_m decreased	T_m decreased	Normal	—	Accelerated	See text
—	+	0	—	—	—	—	—	—	—	

dried at 85 C Suspensions were prepared in Owren s buffer

Streptokinase "Streptase®" (Behringwerke) dissolved in phosphate buffer pH 7 6 Aliquots of 2000 units/ml were stored at −21 C and used just after thawing

Casein Merck, preparation after Hammarsten

Fibrinogen Bovine fibrinogen (Poviet, Holland) clottability approx. 56% The freeze-dried *preparation* was dissolved in isotonic saline and adjusted with distilled water according to the declaration of each batch to give a final concentration of 1.5% clottable protein and ionic strength 0 15 This stock solution was stored at −21 C and diluted with Owren s buffer just before use

Adenosine-d phosphate Trisodium salt of adenosine 5-diphosphate (Boehringer & Sohne) dissolved in Owren s buffer 300 μg/ml stored at −21 C This stock solution was diluted with Owren s buffer before use

Tubes pipettes etc

Blood collecting tubes Five ml polyethylene tubes with polyethylene cap (Heger Plast" Oslo Norway)

Tubes for clotting tests and dilutions Polystyrene tubes 70×10/11 mm and 100×15/16 mm with poly styrene cap (Nunc Ltd Roskilde Denmark) Glass tubes 80×10/11 mm and 100×15/16 mm All tubes were used once only

Petri dishes Disposable sterile acryl dishes 90×1.5 cm (Nunc Ltd Roskilde Denmark)

Pipettes For transfer of plasma after centrifugation

Patient no 5		Patient no 6	Patient no 7	Patient no 8						Normal range or simultaneous control
11 11 66	10 1 67	16 1 67	18 11 66	1 2 67	22 3 67	25 3 67	12 4 67	20 4 67	11 5 67	
0 8	0 46	0 6	0 49	0 54	0 61	0 55	0 53	0 82	0 72	0 2–0 4
10 5/	10/	11/	12/	10 5/	16/	>180/	14/	14/	11/	Patient/
11	10 5	11	10 5	11 5	11	10	11	10 5	10	normal control
					(1 /10)[a]	(12/9)[a]	(12/9 5)[a]	(14/10)		
90	>240	120	>240	>300	> 40	>240	>300	100	>300	>120 mean 300 min
—	0	—	—	—	—	—	0	—	0	0–trace
—	49	—	—	—	—	—	16	—	20	0–50
—	—	152	—	—	—	—	—	—	—	70–120

Table III *Tests for platelet function*

Test	Patient no 1				Patient no 2		Patient no 4		
	22 9 66	8 10 66	9 10 66	12 10 66	3 2 67	3 5 67	7 6 66	7 7 66	30 11 66
Bleeding time min } Right ear	2¾	—	—	3½	1	2½	1¾	7½	3
Bleeding time min } Left ear	2½	—	—	3¾	4	4	1¾	2¼	1¾
Platelets $\times 10^3/\mu l$	226	—	—	223	149	199	141	136	44
Prothrombin consumption index	14	—	—	12	< 6	5	< 4	< 5	< 7
Platelet aggr with ADP } 3 μg/ml	—	—	—	—	13	—	9	—	10
Platelet aggr with ADP } 0 3 μg/ml	—	—	—	—	22	—	15	—	25
Clot retraction 1 h/37 C	—	—	—	—	Normal	—	—	—	Normal
Platelet morphology phase contrast microscopy	—	—	—	—	—	—	Normal	—	Normal
Platelet adhesiveness	—	—	—	—	—	—	—	—	—

disposable polystyrene eye-drop pipettes (Nunc Ltd Roskilde Denmark) were used For volumes of 2 ml and less constriction pipettes (Carlsberg pipettes) were used The pipettes were rinsed in alkaline (pH 9 6) detergent (Triton X" Kelanon, Copenhagen Denmark) tapwater and distilled water and finally dried at 170 C

Cannulas Venipuncture cannulas polished inside (Acu firm V2A 1403/12 Ipo) The needles were used once only

Blood Sampling Technic

Venipuncture was carried out in an antecubital vein or a dorsal hand vein with a minimum of venous stasis 1-2 ml of blood was discarded and the samplings were performed in the followin order and volumes 2×3 ml blood in EDTA glas tubes (80×10/11 mm) for platelet count and red blood cell volume 4×1 ml for whole blood clot ing times 5-6 ml in a 100×15/16 mm glass tube containing 1 ml of glass beads (diameter 2 mm) for preparation of serum and 5×4 5 ml in blood col lecting tubes with 0 5 ml of citrate 3 8 % in each tube The citrated blood wa immediately cooled in ice water and centrifugation was carried out within 0 min after withdrawal Platelet poor plasma was obtained after centrifugation for 5 min at 15 000 rpm (Christ High Speed Centrifuge) Platelet ri h plasma was obtained either after spontaneous ed mentation of the blood for 1-1 / hour or after ent ifugation for 5 min at 1500 rpm (Ecco Superior II B centrifuge) Plasma was transferred to polyethylene tubes with a polystyrene pi pette kept at 4 C until l is soon after or imme diately frozen at − 1 C i zen samples were analysed within four days after sam l ng

Normal control plasma I n pooled plasma from 8-10 normal healthy individua The plasma was frozen at −21 C in 5 ml aliquots pol styrene tubes imme diately after withdrawal an reparation The plasma pool was exchanged at least ry third week

Analytical Methods

Whole blood clotting time One ml of blood was sampled in each of 2 glass tubes (80×10/11 mm) and 2 poly styrene tubes (70×10/11 mm) The tubes were kept at room temperature and tilted once every 15 sec The time for solid clotting was recorded on stopwatches and the mean of duplicate determinations calculated S.D of single determinations calculated by means of duplicate determinations was for glass tubes ±¹/₂ min and for plastic tubes ±2 / min

Kaolin-cephalin time 200 μl platelet poor plasma +200 μl cephalin +100 μl kaolin suspension 4% were incubated at 37 C in a 80×10/11 mm glass tube After exactly 3 min 00 μl $CaCl_2$ 1/40 M was added and the clotting time in seconds was recorded The results are means of duplicates S D of single determinations cal culated by means of duplicate determinations ±0.5 sec For all determinations a simultaneous normal control was run

Thromboplastin time 200 μl platelet poor plasma+ 200 μl thromboplastin prewarmed in a 80×10/11 mm glass tube After exactly 1 min 200 μl $CaCl_2$ 1/40 M was added and the clotting time in seconds recorded. Results are means of duplicates The test included a simultaneous normal control *S.D of single determina-tions* ±0 4 sec

Plasma thrombin time 400 μl platelet poor plasma incubated for exactly 1 min at 37 C in a 80×10/11 mm glass tube The clotting time after addition of 100 μl thrombin solution 10 N I.H units/ml was recorded. Simultaneous normal control S.D of single determina-tions ±0 4 sec

Plasma protamine thrombin time This was carried out just as the thrombin time but the thrombin solution contained protamine sulphate 100 μg/ml

Factors II+VII (p and p-test) was determined by the method of Owren and Aas (2) For preparation of standard curves and analytical control prothrombin

Patient no 5		Patient no 6	Patient no 7	Patient no 8						Normal range or simultaneous control
11 11 66	10 1 67	16 1 67	18 11 66	1 2 67	22 3 67	25 3 67	12 4 67	20 4 67	11 5 67	
9¾	—	3½	> 15	1½	½	2¾	2¾	1¾	> 15	1–5
3¼	—	4½	> 10	1½	5½	3¼	1	—	2	1–5
40	41	184	3380	498	895	1350	1640	531	2100	150–400
11	—	< 5	< 9	< 7	9	—	< 10	< 17	20	0–30
15	—	—	4	—	20	—	7	6	—	7 ± 1 8
25	—	—	6	—	30	—	10	15	—	13 ± 3 0
Slightly reduced	—	—	Normal	—	Slightly reduced	—	Slightly reduced	—	—	
Aniso-cytosis, poikilo-cytosis	—	—	10–15 abnormal platelets	—	40 50% abnormal platelets No spon taneous aggregat	—	20 30% abnormal platelets No spon taneous aggregat	70–80 abnormal platelets. No spon taneous aggregat	90–100 abnormal platelets No spon taneous aggregat	
	—	—	—	—	—	—	28%		29	56 ± 10

standard plasmas "Protrol®" and "Tentrol®" (Pfizer) were used. The results are means of duplicates s.d. of single determinations ±3–4%.

Factor V This was estimated by the method of Wolf (27) with modifications using citrated plasma, Owren's buffer as diluent, and human brain thromboplastin. For each determination a new standard curve of our pooled normal plasma was prepared s.d. of single determinations calculated by means of duplicate determinations was 3.5–4 at the 50–100% level.

Factors VIII (AHG) and IX These were estimated by the kaolin-cephalin time system using plasma from patients with known severe haemophilia A and B as substrate plasma (factor level < 1%) 100 μl substrate plasma + 100 μl patient plasma dilution + 100 μl cephalin + 100 μl kaolin suspension 2% were prewarmed in a glass tube 80 10/11 mm at 37 C Exactly 3 min later 100 μl $CaCl_2$ *1/40 M* was added and the clotting time in seconds recorded The patient plasma was tested in 2 or 3 dilutions (usually 1/10–1/80) with Owren's buffer The results were read in per cent of normal from a standard curve of our normal pooled plasma serially diluted 1/10–1/320 if necessary 1/640 New standard curves were prepared for every 8–10 patients samples All dilutions were kept on ice and prepared in polystyrene tubes The s.d. of single determinations varies with the factor concentration At an AHG and factor IX level of ≤1% the s.d. was ±0.4 and ±0.3% at the 40–60% level ±2.8 and ±3.4% at the 80–100% level ±4.4 and ±5.3% and at the 150–300% level ±23.5 and 39.4 for AHG and factor IX respectively

Thromboplastin generation screening test 400 μl prewarmed Owren's buffer + 500 μl cephalin + 500 μl prewarmed $CaCl_2$ 1/40 M were incubated for 1 min at 37 C in a 100 × 15/16 mm glass tube 50 μl of platelet poor plasma was added At exactly one min intervals from the addition, 100 μl of the incubation mixture was transferred to a series of 80 × 10/11 mm glass tubes containing 100 μl $CaCl_2$ 1/40 M and placed in a waterbath of 37 C Five sec later 100 μl prewarmed normal substrate plasma diluted 1 2 with Owren's buffer was added to the tube and the clotting time recorded on a separate stopwatch The thromboplastin generation curve may for example be characterized by the substrate plasma clotting time in sec after 1 and 3 min incubation (t_1 and t_3) the minimal substrate plasma clotting time in sec (t_m) and the incubation time in min at which t_m is reached (T_m) For each test a normal control test with pooled plasma is run The values of 32 normal individuals during the period of investigation were $t_1 = 116 \pm 10.5$ sec $t_3 = 101 \pm 2.02$ sec $t_m = 15.0 \pm 1.39$ sec and $T_m = 8 \pm 0.8$ min and the mean values of 25 simultaneous controls of our normal plasma pool determined at different days were $t_1 = 1\,0 \pm 13.3$ sec $t_3 = 107 \pm 14.9$ sec $t_m = 15.0 \pm 1.11$ sec and $T_m = 8 \pm 0.6$ min. When a patient sample produced values of t_1 or t_3 and T_m shorter than 2 × s.d. that of the simultaneous control test we considered the test to be "accelerated"

Demonstration of clot accelerators in patient samples To the incubation mixture of the thromboplastin generation screening test 40 μl normal platelet poor plasma and 10 μl platelet poor patient plasma were added simultaneously The test was then carried out in the usual way When the curve of the mixture compared with the curve of normal plasma was accelerated as described above the patient plasma was considered to possess clot accelerating properties.

Thrombin generation test This test was carried out with platelet rich fresh plasma according to the description by Ollendorff (21) slightly modified (Owren's buffer was used as a diluent and as fibrinogen the Poviet preparation was used in a concentration of 0.15 g clottable protein per 100 ml in Owren's buffer) Among other things the thrombin curve is characterized by the time of maximal generation t_m and the maximal thrombin concentration

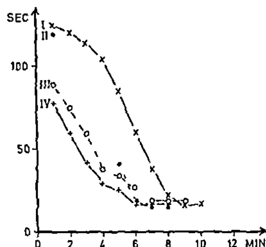


Fig 1 Thromboplastin generation screening test in patient no 1 on the 8th (IV) 9th (III) and 10th (II) of October Curve I is a normal control curve On the 8th and 9th free haemoglobin in plasma was 8 and 23 mg per 100 ml respectively

$$\left(\frac{600}{\text{clotting time in seconds}}\right) T_m$$

Values and S D in 30 normals were $t_m = 6 \pm 1.0$ min and $T_m = 44.9 \pm 19.20$ When t_m was 4 min or less the thrombin generation test was considered to be accelerated

Fibrinogen This was estimated by the method of Jacobsson (14) as modified by Nilsson and Olow (19) The determinations were made in duplicate S D of single determinations 0 02 g/100 ml Antifibrinolytic agents were not added to the blood collecting tubes

Plasminogen The assay was performed by the method of Alkjaersig et al (1) as described by Biggs and Macfarlane (3) The trichloroacetic acid soluble fraction was read in a Beckman DU Spectrophotometer at 290 nm For each test series standards of our normal plasma pool serially diluted with the phosphate buffer pH 7 9 were assayed The test samples were read on a standard curve prepared for each series of tests and expressed in per cent of the normal S D of single determinations 3 1 %

Fibrinolytic activity This was estimated by the *euglobulin clot lysis method* as described by Nilsson and Olow (20) and by testing plasma and plasma euglobulin (prepared as described for the above mentioned method by precipitating plasma with acetic acid at pH 5 4) on *fibrin plates* (?) Bovine fibrinogen (Poviet) was used in a concentration of 0 1% clottable protein in Owren s buffer pH 7 4 ionic strength 0 15 9 ml of fibrinogen was used for each Petri dish and clotted with 250 μl of thrombin 10 N I H units per ml in Owren s buffer Drops of 30 μl of the test solutions were placed on the plates which were incubated for 18–20 hours at 37 C The activity was recorded as the product of two perpendicular diameters in mm of the lysed zone (average of 2 determinations) The fibrinolytic measurements were performed within 30 min after withdrawal of the blood

Bleeding time was determined by the principles of Duke with modifications In each ear lobe a standardized puncture 4×2 mm was made with a Sera Sharp® (Propper Manufacturing Co Long Island City N Y USA) The bleeding points were wiped gently with filter paper until no more blood was seen The S D of a single determination calculated by means of the duplicate determinations is $\pm 1^1/_2$ min

Prothrombin consumption test This was determined essentially as described by Biggs and Macfarlane (3) Serum was obtained after clotting of venous blood in a glass tube with glass beads as described under blood sampling technic Citrated serum was used as described by Biggs and Macfarlane Instead of saline Owren s buffer was used the fibrinogen was Poviet fibrinogen 0 15 g/100 ml in Owren s buffer ionic strength 0 15 The results are means of duplicates

Platelet aggregation with ADP To 250 μl platelet rich plasma in glass tubes 80×10/11 mm was added 25 μl adenosine-di phosphate in Owren s buffer 30 and 30 μg/ml The tubes were rotated at an angle of 15 to the horizontal plane and the time from addition of ADP until macroscopic aggregates appeared was recorded The test was carried out at room temperature The aggregation time was determined with an S D of ±1 sec

Clot retraction This was determined in 500 μl of platelet rich plasma +100 μl thrombin (10 N I H units per ml) after 1 hour at 37 C The patient samples were compared with simultaneous normal controls and expressed semiquantitatively as normal slightly reduced reduced or absent

Platelet morphology Platelet rich plasma was observed between an ordinary glass slide and a cover slip in a phase contrast microscope To evaluate changes in aggregation properties, viscous metamorphosis and other changes during coagulation of the plasma, calcium chloride 1/20 M and platelet rich plasma in the proportion 1 to 2 were mixed on a slide immediately covered and observed in the microscope until the coagulation process was completed

Platelet adhesiveness was determined by the method of Hellem (17) with only slight modifications blood was collected in polyethylene tubes and the sedimentation of the blood dilutions was performed in 75 ml polyethylene tubes S D of a single determination calculated from duplicate determinations was ±4 8%

RESULTS

Table I summarizes the results of the coagulation studies None of the patients had coagulation defects The decreased concentration of factors II+VII and the prolonged thromboplastin time in patient 8 on March 22nd and 25th and April 20th were due to oral anticoagulation therapy On March 25th the patient had also received heparin which was reflected in the prolonged kaolin-cephalin time in the thrombin generation test and in the plasma thrombin time (see Table II)

In contr st several patients showed certain chan es which may indicate activation of their clotting system Thus patients 1 4 5 6 and 8 had short kaolin-cephalin time on one or more occasions Correspondingly the thromboplastin generation screening test was accelerated in the same patients and the activity of factors II + VII was high in patients 1 4 5 and 6 and of factor VIII in patients 1 4 5 6 and 7 The thrombin generation test was accelerated in patients 5 and 8 In patient 7 the thrombin generation was low on February 2nd and June 11th despite normal factors and high platelet count (see Table III) This was probably due to a qualitative platelet defect as the bleeding time was prolonged and the platelet morphology was slightly abnormal

No abnormalities were observed in the fibrinolytic measurements shown in Table II Patient 8 had a high concentration of fibrinogen throughout the period of investigation

The platelet function tests are shown in Table III In patient 5 a clear thrombocytopenia was found and a slightly prolonged bleeding time in one ear which can be explained by the low platelet count In phase contrast microscopy her platelets showed some anisocytosis and poikilocytosis but no definite abnormalities Pa ient 7 had severe thrombocytosis morphologically abnormal platelets and a prolonged bleeding time ADP induced rapid aggregation in his platelet rich plasma and also the clot *retraction* was normal Patient 8 developed severe thrombocytosis during the period of investigation and a large number of his platelets were morphologically abnormal The aggregation with ADP was normal or slightly reduced and the clot *retraction* slightly reduced This last phenomenon and the low platelet adhesiveness to glass beads measured on two occasions may however be a result of the very high platelet number On one occasion on Jun 11th his bleeding time was prolonged in one ear

In all the patients examined during haemolytic crisis evidence of thrombosis was found (patients 1 4 5 7 and 8) Patient 7 had myelofibrosis and thrombocytosis and a possible PNH effect on the coagulation is *difficult to* evaluate In patients 1 4 5 and 6 we tried to demonstrate clotting accelerators in the plasma by means of the thromboplastin generation screening test The results are seen in Table I Fig 1 shows the findings and course in patient 1 It is seen that the patient s plasma induced significant acceleration of the normal generation curve on Oct 8th which was the first day of an episode with increased haemolysis a somewhat slighter acceleration on the following day and no definite acceleration five days later

DISCUSSION

There is general agreement that the thrombotic episodes in PNH patients usually follow periods of accentuated haemolysis The clotting mechanism of PNH patients has been the subject of several studies Mainly three mechanisms have been considered to contribute to thrombus formation

1 An abnormality residing in the platelets which renders them more susceptible to destruction and liberation of thromboplastic material than normal and endows them with increased aggregation properties (7 17 see also 11) This hypothesis however has not been confirmed by *others* (cf 16 9)

2 Liberation of thromboplastic factors from the abnormal erythrocytes either from the intact abnormal erythrocyte (16) and/or primarily from the reticulocytes (4) or due to release of haemolysate (23)

3 Increased activity of clotting factors primarily factors VIII (8) V and VII (15)

Decreased clotting activity probably due to deficiency of factor XI activity was reported by Topp and Hart (25) who concluded that this might be due to consumption by intravascular coagulation This could be induced by liberation of coagulant material from erythrocytes resulting *in thrombin* formation which again accelerates haemolysis and creates a vicious circle With a *very sensitive* clotting system Newcomb and Gardner (18) demonstrated an accelerated *thrombin* generation in PNH patients and suggested that the hypercoagulable state probably was related to the formed elements of the blood With less refined clotting tests changes in PNH plasma could not be detected

Like Newcomb and Gardner (18) we failed to demonstrate any changes or hypercoagulability in the *whole blood* clotting time recalcification time and thromboplastin time With other methods however some abnormalities could be

demonstrated kaolin-cephalin times were shortened thromboplastin generation was accelerated and in two patients thrombin generation was accelerated Like others we could also demonstrate increased activity of the clotting factors II+VII V and VIII in some patients This might be due to a real increase of concentration but was more likely a result of non specific accelerators in the plasma which exerted their effects in the test systems used The accelerating effects of PNH plasma on the thromboplastin generation of normal plasma may also be explained in this way

Our results do not allow any conclusions concerning the mechanism of the clot acceleration in PNH patients However the demonstration of accelerated thromboplastin generation in patient 1 during haemolysis followed by disappearance of the acceleration after the acute haemolytic attack might point to release of clot accelerating substances during haemolysis That not only haemoglobin is responsible seems reasonable as the clot acceleration was less on the second day when free haemoglobin in plasma was at its highest Release of other substances from the lysed erythrocytes for example thromboplastic lipoids (26) has also been proposed (18)

Thrombocytopenia is a frequent finding in PNH patients It has been explained on the hypothesis that PNH platelets undergo lysis more easily than normal platelets Against this hypothesis stands the observation that the platelet life span in PNH patients is normal (6 11)

Lately Regoeczi et al (24) and Brain and Hourihane (5) have drawn attention to the occurrence of haemolysis in the Schwartzman reaction They also demonstrated in rabbits that intravascular coagulation seems to produce haemolysis which can be prevented by heparin Such a mechanism might be a possibility in PNH and the thrombocytopenia could be the result of platelet consumption in intravascular coagulation during the crises If this were the explanation however one would expect to find variations in platelet concentration from time to time One could also expect to find more or less evidence of clotting factor consumption and/or secondary fibrinolysis in these cases This was not found in our patients who were repeatedly studied Only in one patient was a definitely low platelet count observed which was unchanged two months later (patient 5) In this patient however it is interesting to note that the thrombin generation and the prothrombin consumption were normal The thrombin formation was even accelerated despite a very low platelet count which normally results in a low and/or retarded thrombin generation and a poor prothrombin consumption The findings might indicate that some other platelet like activity was present in the patient s plasma

The abnormal platelet morphology and platelet function observed in patients 7 and 8 with myelofibrosis were most likely an effect of an abnormal platelet production resulting in the marked thrombocytosis As mentioned above conclusions concerning the possible PNH effect on coagulation in these patients must be subject to reservations

The definition of clinical hypercoagulability is a matter of debate and laboratory hypercoagulability is an even more doubtful concept Up to now there is no proof that laboratory hypercoagulability (shortened clotting time increased clotting factor concentrations accelerated thromboplastin and thrombin generation and/or increased platelet clumping tendency) indicates an increased thrombotic risk Laboratory hypercoagulability may be observed in patients with clinically manifest thrombotic diseases and also in many severely ill patients with a variety of diseases But it may also be seen in healthy individuals who never had thrombosis The laboratory hypercoagulability may be the reason why PNH patients develop thromboses or the laboratory findings may be secondary to the formation of thromboses Furthermore laboratory hypercoagulability might be an in vitro phenomenon without any significance in vivo The conclusions of our findings must therefore be limited to the observation that in PNH patients a certain laboratory hypercoagulable state" can be demonstrated and that this hypercoagulability increases during the haemolytic crises The studies have not revealed changes in clotting and fibrinolysis indicative of consumption coagulopathy as would be expected during a state of intravascular coagulation

REFFRENCES

1 Alkjærsig N Fletcher A P & Sherry S J clin Invest 38 1086 1959

2. Astrup, T & Mullertz, S Arch Biochem 40 346 1952
3. Biggs R. & Macfarlane R. G Human blood coagulation and its disorders Blackwell Oxford 1962
4. Bradlow B A Brit J Haemat 7 476 1961
5. Brain, M. C & Hourihane D O B Brit J Haemat 13 135 1967
6. Cohen P Gardner F H & Barnett, G O New Engl J Med 264 1294 1961
7. Crosby W H Blood 8 769 1953
8. Egeberg, O Scand J clin Lab Invest 14 217 1962
9. Flexner J M Auditore J V & Hartmann R. C. Amer J clin. Path 33 6 1960
10. Hansen, N E & Killmann S A Acta med scand 184 575 1968
11. Hartmann, R C & Jenkins D E Jr Blood 25 850 1965
12. Hellem, A J. Scand J clin. Lab Invest Suppl 51 1960
13. Hjort, P F Rappaport S I & Owren, P A. J Lab clin Med 46 89 1955
14. Jacobsson, K. Scand J clin. Lab Invest. Suppl. 14 1955
15. Lasch, H G Linke A & Sessner H H Acta haemat. 13 366 1955
16. McKellar M & Dacie J V. Brit J Haemat 4 404 1958
17. Mendel J L Williams M J & Clapp M P Brit J Haemat 2 194 1956
18. Newcomb T F & Gardner F H Brit J Haemat. 9 84 1963
19. Nilsson I M & Olow B Thrombos Diathes. haemorrh. (Stuttg) 8 297 1962
20. — Acta chir scand 123 247 1962
21. Ollendorff P Thrombos Diathes haemorrh (Stuttg) 4 244 1960
22. Owren P A & Aas K. Scand J clin Lab Invest 3 201 1951
23. Quick, A J Georgatsos J G & Hussey C V Amer J med Sci 228 207 1954
24. Regoeczi, E Rubenberg, M L & Brain M C Lancet 1 601 1967
25. Topp J R & Hart G D Canad med Ass J 85 1385 1961
26. Troup S B Reed C F Marinetti G V & Swisher S N J clin Invest 39 342 1960
27. Wolf P J clin Path 6 34 1953

Congress Announcements

The Twenty third Annual Symposium on Fundamental Cancer Research GENETIC CONCEPTS AND NEOPLASIA will be held at The Shamrock Hilton Hotel Houston Texas March 5 to 7 1969

Symposium Registration Office address Texas Medical Center Houston Texas 77025 USA

The Fourth International Congress of Nephrology will be held in Stockholm Sweden June 22 to 27 1969

Organizing Committee

President Professor Nils Alwall M D
Vice President and Chairman of the Organizing Committee Professor Bertil Josephson M D
Vice President Professor Curt Franksson M D
Secretary General Dr Fredrik Berglund M D

Address Fourth International Congress of Nephrology P O Box 272 S 101 23 Stockholm 1 Sweden

The Second International Symposium on Gastrointestinal motility will be held in Rome September 10 to 14 1969 The programme will include a limited number of reports followed by discussion and a Round Table The English language will be used throughout Informations from Dr Doc Aldo Torsoli 2 Clinica Medica dell Universita Viale del Policlinico 00100 Rome Italy

The Seventh International Congress of Diabetes will be held in Buenos Aires August 23 to 28 1970

President V G Foglia M D Address Seventh Congress International Diabetes Federation Paraguay 2155 7th Floor Buenos Aires Argentina

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